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Abstract

Since Louis de Broglie introduced the concept of matter-waves nearly a century ago, the wave nature of matter has been confirmed in numerous interference experiments. Talbot-Lau interferometers with optical gratings have proven particularly effective for enabling single-particle interference experiments with complex molecules. Furthermore, matter-wave interferometers have been used to measure molecular properties such as polarizability, magnetic moment, or susceptibility. A particularly interesting class of particles are biomolecules such as polypeptides and proteins. Studying proteins in the gas phase is uncharted territory, and their investigation in interferometers would be a first step in this direction.

Three main challenges arise in this context, the generation of a neutral protein beam, diffraction in the Talbot-Lau interferometer, and the detection of proteins by photoionization. This work explores how photocleavable tags (PCTs) can be used to overcome these difficulties. PCTs enable the controlled cleavage of a chromophore-containing molecule from a protein following the absorption of a photon, which can alter the charge state.

This thesis discusses, how PCTs can be used to facilitate the neutralization, diffraction, and detection of proteins in a pulsed interferometer. Experiments that involve PCTs are discussed, and potential tag candidates are introduced.

Molecular beams of PCTs are generated with the technique of matrix-assisted laser desorption/ionization. An optical parametric oscillator is additionally used to investigate their photochemical properties and characterize their photon absorption behavior. This is enabled by the study of mass spectra acquired in a time-of-flight mass spectrometer.

Zusammenfassung

Seit Louis de Broglie vor fast einem Jahrhundert das Konzept der Materiewellen einführte, wurde die Wellennatur der Materie in zahlreichen Interferenzexperimenten bestätigt. Talbot-Lau-Interferometer mit optischen Gittern haben sich als besonders effektiv erwiesen, um Einzelpartikel-Interferenzexperimente mit komplexen Molekülen zu ermöglichen. Darüber hinaus wurden Materiewellen Interferometer verwendet, um molekulare Eigenschaften wie Polarisierbarkeit, magnetisches Moment oder Suszeptibilität zu messen. Eine besonders interessante Klasse von Teilchen sind Biomoleküle wie Polypeptide und Proteine. Die Untersuchung von Proteinen in der Gasphase ist Neuland, und ihre Untersuchung in Interferometern wäre ein erster Schritt in diese Richtung. In diesem Kontext ergeben sich drei Hauptprobleme, die Erzeugung eines neutralen Proteinstrahls, die Beugung im Talbot-Lau-Interferometer und die Detektion von Proteinen durch Photoionisation. Diese Arbeit untersucht, wie Photocleavable Tags (PCTs) verwendet werden können, um diese Schwierigkeiten zu überwinden. PCTs ermöglichen die kontrollierte Spaltung eines chromophorhaltigen Moleküls von einem Protein nach der Absorption eines Photons, was den Ladungszustand ändern kann.

In dieser Arbeit wird diskutiert, wie PCTs verwendet werden können, um die Neutralisation, Beugung und Detektion von Proteinen in einem gepulsten Interferometer zu erleichtern. Es werden Experimente besprochen, die PCTs involvieren, und potenzielle Tag-Kandidaten vorgestellt.

Molekularstrahlen von PCTs werden mit der Technik der Matrix-unterstützten Laserdesorption/Ionisierung erzeugt. Ein optischer parametrischer Oszillator wird zusätzlich verwendet, um ihre photochemischen Eigenschaften zu untersuchen und ihr Photon Absorptionsverhalten zu charakterisieren. Dies wird durch die Untersuchung von Massenspektren ermöglicht, die in einem Flugzeit-Massenspektrometer erworben wurden.

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Chapter 1

Introduction

Since the pioneering works from the beginning of the 20th century, quantum physics has transformed our understanding of nature's fundamental processes in the most radical way. The theory has been extraordinarily successful in explaining experimental observations in various fields of physics, be it by correctly interpreting the atomic spectral lines and explaining the structure of the periodic system, properties of condensed matter, predicting wave-particle duality, or in the enhanced and most sophisticated formulation of Quantum field theory, building a theory of nature's most fundamental constituents and unifying three of the four fundamental interactions in the standard model. Specifically, transitions in $^{27}\text{Al}^+$ were used to build atomic clocks with a systematic uncertainty below $< 10^{-18}$ [1]. The hydrogen 1S - 2S transition frequency was determined with a fractional frequency uncertainty $\Delta f/f$ on the order of 10^{-15} . Measurements were conducted that ascertain the fine structure constant α with a relative accuracy of 81×10^{-12} [2], and the electron g_2 -factor with an accuracy of 1 part in 10^{12} [3]. These are examples of the most precise empirical verifications for a scientific theory in human history.

Despite this overwhelming success, the theory is still widely considered to be counterintuitive. The reason for that is mostly attributed to the fact that characteristic quantum phenomena implied by the mathematical formalism, like the superposition principle, interference and entanglement are typically not encountered on the macroscopic scale and this absence has prevented our perception of nature to evolve to a point, where we can build an intuitive understanding of these concepts. However, quantum theory is not a specialized theory of the microscopic, but declares to be a fundamental theory of nature, with classical physics being merely an approximation. Furthermore, quantum physics is already ubiquitous in the modern world, however just in a veiled way [4]. Understanding quantum laws enabled the development of the transistor, ubiquitous in the age of information. Nuclear magnetic resonance, a widely established medical diagnosis technique would not be possible without understanding nuclear spin, a non-classical concept. The quantum tunnel effect plays an indispensable part in the process of nuclear fusion in the sun, laser scanners are used in every grocery store and the list goes on. However, these processes all happen somewhat behind the curtain and the above-mentioned effects can only be directly demonstrated in the physicist's lab, under very specific and precarious conditions.

Particularly, in our labs at the University of Vienna, we are interested in verifying and demonstrating the quantum wave nature of massive and complex particles in specialized matter-wave interferometers to test quantum physics with mesoscopic bodies and over mesoscopic distances. More precisely, we want to explore if the superposition principle and delocalization of a matter-wave implied by the Schrödinger equation (see chapter 2) can be extended to particles of higher masses and internal complexity. Even if quantum mechanics is fundamentally true, its characteristic phenomena may be suppressed by interaction with the environment, as described by the theory

of decoherence [5–7]. In this case, it would be a technical challenge to shield our systems from the environment, which becomes more and more difficult with the size of the systems. Regardless, this does not stop us from seeing how far we can push it, which is what we are trying to do in our group, by building on matter-wave research that has experimentally verified the wave nature of matter in a long history of experiments with different particles like electrons [8, 9], neutrons [10–12], atoms [13–15] and various molecules [16–19], to name a few, some of which will be more closely examined in chapter 2. In addition to probing fundamental principles of quantum physics, the sensitivity of interferometers allows innovative measurements of molecular properties and phenomena. Specific examples include the measurement of molecular absorption cross sections [20], molecular polarizability [21] and diamagnetism [22]. Interferometry with atoms was used by other teams to test fundamental aspects of gravity like the equivalence principle, the gravitational constant, gravitational acceleration and gravity induced quantum decoherence [23–26].

Our group continues to further develop these techniques and has already succeeded in constructing interferometers that have demonstrated the quantum wave nature of metal clusters with masses beyond 25 kDa [27]. While the group is also pushing towards high mass interference using metal clusters, the goal of this master thesis is to open the door to construct an interferometer that works with biological material, specifically proteins. The quantum wave nature of a 15 amino acid polypeptide has already been successfully demonstrated [28] (section 2.4). We want to build upon this and advance to the smallest protein insulin and beyond as a next step. Proteins are fundamental constituents of all biological processes in complex life forms. Establishing that they too adhere to the principles of quantum mechanics would be a remarkably intriguing discovery. In addition, the investigation of isolated proteins in a gaseous state represents a largely uncharted territory. Measurements on proteins with interferometers could yield insights into attributes such as polarizability, functional dependence, magnetic moments, susceptibility, protein folding, and their overall internal structure. This could represent a substantial stride towards the convergence, or at the very least, enhancing the intersection of biology and quantum physics.

The thesis is divided into six chapters. Chapter 2 serves as motivation and starting point for the following chapters. It provides a brief insight into the complex topic of interferometry, discussing past experiments and interferometer concepts to derive the essential components for a potential protein interferometer. It will become apparent that three key components are necessary: a stable source for a protein beam, a diffraction mechanism, and a detection scheme. Chapter 3 then dives into potential source techniques, specifically the desorption of molecules with ultra-short laser pulses, distinguishing between pure laser desorption (LD) and matrix-assisted laser desorption (MALDI). Chapter 4 addresses the main topics of our investigation, photocleavable tags (PCTs). Roughly speaking, PCTs are smaller molecules containing photolabile protecting groups, which attached to proteins, could allow control of the protein's charge state upon photon absorption. As will be shown in chapter 4, this could facilitate the neutralization, diffraction, and detection of proteins necessary for the interferometer, which is to date not feasible with conventional methods. The investigation of potential source mechanisms and testing photochemical properties of PCTs for charge state control is the main scope of this work. The experimental setup used for that purpose will be discussed in chapter 5. The final chapter concludes with an outlook.

Chapter 2

Matter-wave Interference

Louis de Broglie introduced the concept of matter-waves [29]. He attributed a wavelength to massive particles, now referred to as the de Broglie wavelength of particles. In the non-relativistic case, i.e., for particles with velocities much smaller than the speed of light ($v \ll c$), the de Broglie wavelength is given by the Planck constant h , divided by the momentum p of the particle:

$$\lambda_{dB} = \frac{h}{p} = \frac{h}{mv} \quad (2.1)$$

where m is the mass of the particle and v is its velocity. The hypothesis of wave properties of particles is the flip side of a coin that is sometimes referred to as wave-particle duality. Phenomena like superposition, interference and diffraction are typically associated with waves. By contrast, particle like behaviour is characterized by a well-defined position and momentum state that follows a precise trajectory and the fact that particles collide with each other and exhibit phenomena like scattering. Wave-particle duality states that quantum objects can demonstrate wave-like or particle-like properties that depend on the specific experiment. The idea that waves can possess particle-like properties had already been pointed out by Albert Einstein in his famous work on the photoelectric effect [30]. The concept of matter-wave was later formalized by Erwin Schrödinger in his wave equation [31].

In the modern formulation of quantum mechanics, a state of a physical system is represented by an abstract vector in Hilbert space $|\Psi\rangle$. The position of a particle is estimated by a probability distribution according to Born's rule: $|\langle \mathbf{x} | \Psi \rangle|^2 = |\Psi(\mathbf{x})|^2$. The rule specifies the likelihood that the particle can be detected at a certain location during a measurement. The complex number $\langle \mathbf{x} | \Psi \rangle$ encodes the position probability, i.e., the amplitude and phase of the translational motion (usually the center of mass) of a physical system like an atom or molecule. Manipulations of quantum states with respect to their phase are in analogy to wave optics and referred to as *coherent* manipulations. A high degree of coherence between different quantum states is a necessary condition for interference effects to be detected. The main task of matter-wave interferometers is to coherently manipulate states of particles and let them interfere, thereby demonstrating their quantum nature. To highlight the similarity with classical light optics, consider the particle version of Young's double slit experiment [32] with electrons at the double slit. The interference effect can be illustrated as follows: According to the superposition principle, the sum of two wave functions is again a solution to the Schrödinger equation. In the case of the double slit, the two wave functions ψ_1 and ψ_2 represent the particle going through either the first or the second slit. The probability to find the particle after the slit is given by the absolute value squared:

$$|\Psi(x)|^2 = |\psi_1(x) + \psi_2(x)|^2 = |\psi_1(x)|^2 + |\psi_2(x)|^2 + Re(\psi_1(x)\psi_2^*(x)) \quad (2.2)$$

The last term in Eq. 2.2 is the interference term since it depends on the relative phase between the complex ψ_1 and ψ_2 . The electron is detected at a precise spatial location, the likelihood

of detecting the particle at this location is governed by the interference of the relative phases. Equation 2.2 is intended to serve as an illustration of interference effects and is based on the formulation of quantum mechanics through the (non-relativistic) Schrödinger equation. Alternatively, rigorous theoretical formulations can be found in Feynman path integral formulations [33] or in descriptions through Wigner functions [34], which directly describe the development in phase space. Relativistic effects are taken into account in [35].

The above case is an example of interference with translational states of a particle. In the following, this will be our main concern. One should emphasize, however, that interference effects can generally occur if a physical system occupies superposition states that are *indistinguishable*, which includes their internal and external degrees of freedom. In fact, long-lived superpositions have historically been first created with internal states of atoms and molecules. Rabi [36] established a method to change the internal state of atoms and further molecules by using oscillating electromagnetic fields and resonant transitions to coherently control spin states and also excited electronic states of resonant atoms on a general note. This concept is now known as Rabi oscillations. Ramsey [37] built on this idea and used two spatially separated resonant radio frequency fields to prepare long-lived superposition states and provide a subsequent readout of the final state, the result depends on the relative phase between the states.

Ramsey's experiment can be regarded as an interferometer for internal states, formally very similar to the optical Mach-Zehnder (MZ) interferometer, where light is first split into two different paths which are subsequently recombined after free evolution, with optional phase interactions in one arm. In Ramsey's version, first an initial state is selected by a Stern-Gerlach apparatus. The beam splitter is realized by the first microwave pulse. After free evolution, the second microwave pulse is used to read out the state, which acts as the recombination mechanism. This experiment can be regarded as the first atom interferometer [38], although it is sometimes put into the category of resonant Bloch vector manipulations. An improved version is the Ramsey-Bordé interferometer [39], that resembles the MZ-configuration even more directly. It uses four pulses, one to split the beam, two subsequent pulses to redirect the particles back together (mimicking the role of the mirrors in the MZ-configuration) and a final one to recombine the beam.

Internal state manipulation may be used for atoms with accessible transitions, for more complex particles with dozens of internal rotational, vibrational, electronic, and spin states, creating a beam of molecules with total indistinguishability between the individual particles is only possible if they can be put into the conditions of a Bose-Einstein condensate. Instead, we can demonstrate single particle interference with the continuous position and momentum states $|\mathbf{x}\rangle$ and $|\mathbf{p}\rangle$ of phase space, by splitting the wave function of a particle into indistinguishable paths along the interferometer. The crucial condition is that the wavelets emerged from the same state in phase space, accumulate phase differences along the way, and are overlapped again at a specific point in time and space. An interferometer for translational states for any type of particle needs to fulfill the following requirements, as has been summarized in [38]:

- (i) Initial state preparation
- (ii) Coherent splitting of the wave function in at least two distinct locations in spacetime
- (iii) Wavelets accumulate phase differences due to different path lengths, external perturbations, and obstacles
- (iv) Recombination of the wavelets
- (v) Detection and readout of the final state by observing the population differences in interference fringes

2.1 Beam preparation

An important prerequisite for the source is to have, to some extent, the particles in a well-defined initial translational state. This process is fundamentally limited by the uncertainty relation $\sigma_x \sigma_p \geq \hbar/2$, where $\sigma_{x,p}$ denotes the standard deviation for position and momentum. Over the past decades, a vast range of techniques have emerged for trapping and cooling of particles of different sizes. We look at a few important examples that have been applied as sources in interferometers.

Evaporative sources and supersonic expansion

Volatile atoms and molecules with high vapor pressure and low boiling points can be launched in effusive and supersonic sources [40, 41]. Effusive beam creation is the evaporation of samples in a Knudsen cell, that is a cylinder wrapped in a heating wire with a small slit to eject the molecular beam. They have been successfully applied to matter-wave experiments and create thermal beams that follow a Maxwell-Boltzmann distribution to a good approximation [42]. The cell can be viewed as a reservoir R that ejects Atoms or Molecules through a small nozzle A with diameter a into the vacuum. For low pressure and density in R , the mean free pathway for particles is high compared to a and collisions can be neglected in the gas. The situation is different for supersonic beam expansion. Supersonic beam creation may be viewed as the rapid expansion of a gas through a nozzle with high pressure and density and a small free path length compared to the opening diameter a . Many collisions take place during expansion through the opening, on a timescale so short that no heat is exchanged and the gas expands adiabatically [43]. The result is a cooling effect that can be illustrated as follows: for a mol of the gas with molar mass M , the total energy is the sum of the internal energy $U = U_{kin} + U_{rot} + U_{vib}$, compression pV and kinetic energy of the fluid $1/2Mu^2$, where u is the velocity of the fluid. Energy conservation demands [43]:

$$U_0 + p_0V_0 + 1/2Mu_0^2 = U + pV + 1/2Mu^2 \quad (2.3)$$

for the total energy before and after expansion, where the 0 subscript labels the quantities before expansion. R is in thermal equilibrium and therefore $u_0 = 0$. The pressure after the expansion is small and can be neglected: $p = 0$. Equation 2.3 then tells us that the increase in the kinetic fluid energy $1/2Mu^2$ comes at the expense of U and the gas can be cooled to temperatures below < 1 K. Another point of view is that the relative velocities in the gas are reduced by the velocity transfer from faster particles to slower ones, until no collisions are present because of the rapid reducing density. The cross sections for the transfer of U_{rot} and U_{vib} in translational energy are generally smaller, temperatures can be assigned in the following order: $T_{kin} < T_{rot} < T_{vib}$ [44]. The low translational energy favors the formation of weakly bound Van der Waals clusters through collisions with a third partner (this includes the walls of R as well). The binding energy released creates a heating effect in return. The number of collisions between sample particles can be reduced by dilution of the sample in a seed gas like argon, however there are now more total collisions which can dissipate the relative energy of the sample particles to the collision partners. Fine tuning of p_0 , T_0 , a and the concentrations of seed gas and sample allows experimental control over temperatures T_{rot} and T_{vib} and the formation of aggregates [43]. Supersonic sources were used in various interferometer experiments with atoms like sodium [14, 15] and different molecules [45–47].

Laser cooling and trapping

Methods based on optical laser cooling and on radiation pressure [48], optical molasses [49] and magneto-optical traps [50] are additionally used to cool and trap atoms for initial state prepa-

ration. Cooling a particle in the field of a laser with angular frequency ω requires a resonant two-level transition for photon absorption $|i\rangle \rightarrow |k\rangle$. The photon transfers momentum upon absorption. Spontaneous emission can occur but randomly in all directions and therefore averages out, with an effective momentum transfer into the propagation direction of the photon of $\mathbf{p} = \hbar q \mathbf{k}$, where q is the number of absorbed photons and $\mathbf{k}$ is the wave vector of the photon. Real atoms are multi-level systems, but approximate two-level systems can be realised by optically pumping them into a state where a closed two-level transition is possible. An example is the hyperfine transition $^2S_{1/2}(F'' = 2) \rightarrow ^2P_{3/2}(F' = 3)$ in Na, the $F'' = 2$ state can be pumped by σ^+ polarized light [43]. Overlap with other hyperfine states is still possible, but they can be pumped out again by an additional pump laser. For molecules this technique is not possible since the excited electronic state typically decays into a different rotation-vibrational mode (J, ν) that is not anymore resonant with the laser. Cooling in all three spatial dimensions is experimentally achieved by an optical molasses, where six slightly off-resonant counter-propagating laser beams in the $\pm x, y, z$ directions are used to cool atoms in all three spatial dimensions. Absorption is increased for negative detuning $\omega_A - \omega_L = \delta\omega < 0$ for atoms that travel anti-parallel to the laser beam, due to the enhanced resonance caused by the Doppler shift of the laser frequency, and therefore receive a net force backwards. It is important to note that this setup does only realise confinement in velocity space, but atoms are not trapped inside the molasses and can diffuse out of the laser field volume [43].

Trapping can be realised in a magneto-optical trap (MOT) with an additional magnetic field $\mathbf{B}$ that splits atom energy levels according to the Zeeman shift $\Delta E_i = -\boldsymbol{\mu} \cdot \mathbf{B} = g_F \mu_B m_F$, where μ_B is the Bohr magneton, g_F is the Landé-factor and m_F is the projection of the total angular momentum $\mathbf{F}$ in the direction of the magnetic field. The field is created by two anti-parallel Helmholtz coils and can be approximated by (considering the 1D case) $\mathbf{B} = bz$ in the near area of $z = 0$ [43]. Two negative detuned ($\omega_L - \omega_0 < 0$) laser beams propagate in the $\pm z$ direction with polarizations $\sigma^\mp$. Consider the transition between $F = 0 \rightarrow F = 1$. At $z = 0$ absorption by both laser beams averages out, if an atom is moving in the $+z$ direction σ^- light is absorbed more (the detuning is smaller than for σ^+ light in the $m_F = -1$ case) and the atom is pushed back. The situation is reversed for the $-z$ direction, with enhanced σ^+ absorption. The principle is applied to all 3 spatial dimensions to trap atoms at the field-free center of the trap. Laser-cooled metastable neon atoms were for example launched from a MOT onto a double slit interferometer in [51]. MOTs can be used as a precooling stage for Bose Einstein condensates (BEC) [52]. After the MOT, they are loaded into a pure magnetic trap and cooled to a BEC by *evaporative cooling* [53]. Interferometers for BECs have been realised experimentally [54–56].

Protein sources

For atoms, effusive sources and laser cooling schemes are well established. For proteins however, efficient trapping or laser cooling schemes are still in early development. Effusive sources are not suitable for non-volatile proteins and optical cooling is difficult if not impossible due to the large number of internal states. A better solution is to rely on soft-volatilization methods developed for mass spectrometry, to create charged and neutral beams of non-volatile molecules. In the field, two methods have proven themselves particularly suitable for this purpose, enabling analysis of heavier proteins up to the regime of whole cells (10^{10} Da) [57], *matrix-assisted laser desorption/ionization* (MALDI) [58] and *electrospray ionization* (ESI, chapter 3) [59, 60]. MALDI in particular, although producing fast and hot beams, could form the basic foundation for the source of a potential protein interferometer. We will therefore take a closer look at MALDI as well as laser desorption without a matrix (LD) in section 3.1.

An interesting alternative to MALDI that should be mentioned is laser induced acoustic desorption (LIAD) [61].

LIAD enables mass analysis of biological samples like vaccinia viruses, *E. coli* K-12 bacteria and human red blood cells [62], that cover a mass range from 10^9 to 10^{14} Da, with a size range from 200 nm to 10 μm . In [62], test particles were put on a bare Si(100) wafer with 0.5 mm thickness that was irradiated with a frequency doubled Nd:YAG laser at 532 nm from the back-side. The laser ablation creates acoustic waves in the wafer that desorb the particles. They are subsequently trapped in a quadrupole ion trap (QIT), the charge is provided by the LIAD-process itself, with no necessity for secondary ionization. Although ionization efficiency is reported to be low, 'precharged' particles can be desorbed and trapped in the QIT, with charge distributions ranging from several 100 charges up to 2000 charges for red blood cells. The authors state that ionization with charge numbers that high is unlikely to occur during acoustic desorption and that there must therefore exist a small amount of precharged particles in the sample. Trapped particles were subsequently illuminated by an argon ion laser for detection by a CCD-camera via elastic light scattering. LIAD can be advantageous for mass spectrometry, since it only requires single particles for mass analysis and does not require the sample to be embedded in a matrix like in MALDI, which reduces background noise and fragmentation. The technique is especially suitable for micron-sized cellular organelles since they can be charged by secondary electron bombardment and detected by elastic light scattering [62]. Other studies with LIAD demonstrated that velocities of the desorbed particles are very low, for example for rhodamine B (479.02 g/mol), an average velocity of 59 ± 12 m/s could be measured [63], which is sufficiently lower than common MALDI-velocities for the same mass range (see experiments in chapter 5). As we will see in section 2.5, small velocities are desirable for masses above 20-60 kDa, however, an interferometer source also necessitates a well-collimated beam with a strong signal. LIAD has not demonstrated the ability to meet these criteria. Nevertheless, if the aim is to expand interferometry to encompass viruses and cells, LIAD could still be an option to consider.

2.2 Detection

Before we look at diffraction mechanisms, it is useful to anticipate and examine established detection techniques used in matter-wave experiments. For this discussion, we follow the overview given in [42]. Detection schemes can roughly be divided into two categories: detection by *ionization* and *surface-based* detection.

Ionization techniques

Molecules can be ionized by *electron impact ionization*, *ionization on a hot surface*, *delayed thermal ionization*, *field ionization*, and *single/multi-photon ionization*.

Electron impact ionization (EI) bombards the targets with electrons to ionize them, with typical energies between 40-100 eV [64]. EI offers typically higher cross sections σ_{ion} than photon ionization, around 10^{-16} cm² up to 10^{-14} cm², but the technique is not considered to be a soft ionization technique and fragments complex molecules. It is therefore not suitable for proteins.

Ionization on a hot surface can occur when the work function of the surface is higher than the ionization limit of the molecule. The molecule can therefore gain energy and, if the surface is hot enough, re-desorb as an ion that can subsequently be detected. Although successfully applied to insulin [65], a necessary condition for the effect to occur was a sufficiently high particle velocity, making the de Broglie wavelength too short for high-mass matter-wave experiments.

Thermal ionization by laser light can be applied to thermally stable molecules like fullerenes [16, 66, 67]. Internal vibrational and rotational degrees of freedom can be excited and energetically couple to electronic states. This leads to competing processes like thermal radiation, ionization,

and fragmentation. The statistical nature of the thermal coupling can delay ionization to many microseconds after the laser pulse. Proteins, however, are thermally too unstable for that process. **Field ionization** uses a strong electric field close to a point surface, like a small tip. Electrons can tunnel from the molecule to the surface to ionize the particles. This technique would be soft enough, but 2D-imaging of the density pattern would be very inefficient because the interaction region around a small tip is limited to about 200 nm.

Fluorescence detection in free flight is an option for particles with narrow transitions; however, for proteins that lack closed fluorescence transitions this is not suitable.

Finally, molecules ionize if the photon energy $h\nu$ is high enough. **Resonant or non-resonant multi-photon ionization** (section 4.1.2) can do the trick for lower energies, if the internal relaxation times are longer than the time between two subsequent absorbed photons. Although photon absorption cross sections are usually smaller (approximately 10^{-17} cm²) than for electron impact ionization, it can still be the better alternative since a high photon flux can lead to more efficiency and it is a softer method due to significantly lower photon energy. Furthermore, photoionization as a diffraction mechanism for interferometry has been already used in experiments (section 2.3.2), so the same mechanism can also be used for detection. Photoionization works well for metal clusters; for proteins ionization thresholds are higher than 8 eV [68] and therefore no more in the range of commercial laser systems. Processes like electron recapture, fragmentation, and re-neutralization increase the difficulty of photoionization. As already mentioned, a possible way to overcome this is by tagging a protein with a small molecule having an accessible ionization threshold, more on this in chapter 4. One could also design artificial biopolymers, with a number of photon-absorbing constituents like tryptophan¹, as has been done in [70], but the first approach is applicable to a wider class of samples.

Surface techniques

Surface-based detection techniques usually apply some form of microscopy; important examples are *scanning tunneling microscopy* (STM) and *fluorescence microscopy*.

STM of molecules deposited on a surface offers probably the highest possible resolution of all techniques discussed and has been successfully applied to fullerenes [71]. It requires appropriate surfaces for the molecules to bond to, and vacuum requirements are reported to be very demanding, around 10^{-10} mbar. Additionally, acquisition times are slow, with around 45 minutes for an area of 2 μm^2 .

Superconducting bolometers are an intriguing possible technique for particle detection in matter-wave experiments, which in contrast to TOF mass spectrometers, are not sensitive to the mass, but to the energy of the incident particle. Although still a topic of research, *superconducting single-particle detectors* (SSPDs) [72] and *superconducting nanowires* (SNW) could prove to be potential interferometer detectors for the future. Unlike conventional mass spectrometry, they do not require exorbitant voltages (see section 3.1) for high masses and could also detect neutral particles, which would facilitate the interferometer design, especially for proteins. We will bring some attention to them in the outlook, chapter 6. For now, we focus our attention on the main component of a matter-wave interferometer, the diffraction mechanism.

2.3 Matter-wave diffraction on transmission gratings

The heart of any interferometer is the beam splitter mechanism that creates a coherent superposition of the wave function. We will focus on diffraction here. Diffraction of matter-waves at a grating is a well-known effect from optical light waves. Diffraction occurs when a wave function's

¹Tryptophan is the only amino acid that can be efficiently ionized, with an ionization energy ≤ 7.5 eV [69].

amplitude and phase are locally shifted by an obstacle, which can lead to interference and patterns of high and low particle density, similar to bright and dark spots for light waves. Matter-waves can be diffracted on various structures; a critical condition for interference is sufficient coherence of the wave, a well-defined phase relation or correlation of points in time and space. Transverse or spatial coherence describes correlations between different points in space in the transverse direction of the beam. The van Cittert-Zernike theorem [73] quantifies transverse coherence and states that the spatial coherence function of an incoherent source is equal to the Fourier transform of the (complex) amplitude function of the source or aperture function. Longitudinal or temporal coherence quantifies correlations between different points in time and depends on the spectral density of the source. The Wiener-Khinchin theorem [74] states that longitudinal coherence is equal to the Fourier transform of the spectral density and therefore depends on the velocity distribution [75]. A diffraction mask spatially modulates the amplitude and/or phase of the wave function. In the early days, atomic gratings have diffracted electrons ([8] on celluloid film, [9] on nickel crystals), or atoms and small molecules ([13] H₂ and He-atoms on a lithium fluoride crystal). Further advancements in nano and laser technology led to the development of *transmission* gratings, consisting of slits with periodicity d in a nanomechanical mask or laser beams that are reflected into each other, creating a standing light wave that acts as a grating with the slits at the position of the nodes (see section 2.3.2). Diffraction on three optical depletion gratings will be the essential mechanism for a protein interferometer and the next sections discuss diffraction effects on mechanical and light gratings and which type of interferometers one can build with them.

Kirchhoff's diffraction theory [76] describes the diffraction of waves at an arbitrary aperture. An illustrative example is the diffraction of a monochromatic plane wave $E(\vec{x}, t) = E_0(\vec{x}) \exp(ikz - \omega t)$ propagating in the z -direction, with angular frequency $\omega = 2\pi c/\lambda$ and wave number $k = 2\pi/\lambda$, on a stationary aperture. The spatial part $E(\vec{x})$ of $E(\vec{x}, t) = E(\vec{x})T(t)$ is governed by the Helmholtz equation, which is formally equivalent to the Schrödinger equation [74]:

$$(\nabla^2 + k^2)E(\vec{x}) = 0, \quad (2.4)$$

$$(\nabla^2 + k_{dB}^2)\psi(\vec{x}) = 0, \quad (2.5)$$

where $k_{dB} = 2\pi/\lambda_{dB}$. Consider the diffraction of the spatial plane wave on an aperture $S(\eta, \xi)$ that lies parallel to the (x, y) plane. Huygens' principle implies that the field at a point $\vec{x}$ after the aperture is the sum of individual wavelets that emerge from the aperture:

$$E(\vec{x}) = -\frac{i}{\lambda r} \iint_S e^{-ikr} E(\eta, \xi) d\eta d\xi. \quad (2.6)$$

This integral can be simplified under the assumption of the paraxial approximation $r \approx z$ and a Taylor expansion up to the second order:

$$E(\vec{x}) = -\frac{i}{\lambda r} \iint_S \exp\left(-ik \left[\frac{-x\xi - y\eta}{z} + \frac{\xi^2 + \eta^2}{2z} \right]\right) E(\xi, \eta) d\eta d\xi. \quad (2.7)$$

The integral in this form is known as the Fresnel approximation or near-field regime. For larger z distances, the quadratic terms in Eq. 2.7 can be ignored. The intuitive physical reason for this is that wavefront curvature decreases with increasing distance from the aperture. This case is known as the Fraunhofer approximation or far-field regime. Setting up a coherent particle source in front of a diffraction grating and a suitable particle detector is, in principle, enough to build a far-field interferometer. The far-field limit in this setup is reached when the distance z is much larger than a^2/λ_{dB} , where a , the aperture size, restrains the beam size [77]. At the grating, single-particle interference creates a fringe pattern of high and low particle density further downstream, the far-field interference pattern with separated diffraction orders. The different diffraction orders

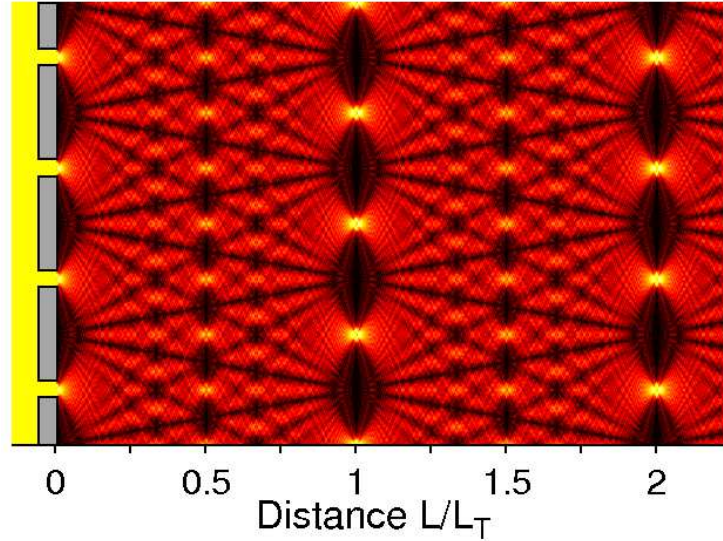


Figure 2.1: Near-field Talbot carpet behind a grating after illumination by a plane wave from the left. High amplitude is indicated by bright colors. The self image of the grating is shifted by half a grating period for the Talbot length $L/L_T = 1$. Unshifted images appear at even multiples of the Talbot length and fractional images at rational fractions of the Talbot length. The picture was extracted from [75]. The calculation was done in the paraxial approximation.

n are determined by $\sin(\theta) = n\lambda_{dB}/d$ [75]. In reality, one usually has to deal with incoherent sources. The transverse coherence behind an incoherent source at a distance L can be estimated by [75]:

$$X_c \approx 2L\lambda_{dB}/a > d, \quad (2.8)$$

where a is the effective size of the source. X_c has to be greater than d to provide sufficient coherence, implying that for more massive particles, one either needs longer travel distances L or even smaller aperture sizes a . As mentioned, the longitudinal coherence depends on the velocity distribution and is therefore an indication of the similarity of the de Broglie wavelengths with respect to the velocity. For a particle cloud with a velocity distribution of $v/\Delta v$ ($\Delta v = \text{FWHM}$), the Wiener-Khinchin theorem implies an approximation:

$$L_c \approx \frac{\lambda_{dB}^2}{\Delta\lambda_{dB}} = \lambda_{dB} \frac{v}{\Delta v} > n\lambda_{dB}, \quad (2.9)$$

for the n^{th} diffraction order to be visible in the far-field. It follows that sufficient longitudinal coherence can be provided by collimating the beam to cut off transverse velocity, or selecting different velocity classes by techniques like gravitational selection or rotating disk choppers [42]. The quantity $2L\lambda_{dB}/a$ illustrates a limitation of far-field experiments. Smaller aperture sizes imply less particle flux and therefore a weaker signal to work with. For experiments with more massive particles in tabletop experiments with limited length L , this approach quickly becomes unfeasible. These coherence limitations can be overcome in the near-field regime.

2.3.1 Talbot-Lau interference

A well-known effect from classical optics in the near-field is the *Talbot effect* [78]. When a diffraction grating is illuminated by a coherent plane wave, Huygens' principle implies the emergence of a diffraction pattern that can be decomposed into a sum of spherical waves, each wavelet coming from a slit of the grating. This is mathematically formalized by calculating the Kirchhoff-Fresnel

integral in the Fresnel approximation. The curvature of the spherical wavefronts cannot be neglected in the near-field. The result is a coherent self-image of the diffraction mask known as the *Talbot carpet* (Figure 2.1), as a result of near-field interference of all diffraction orders. The pattern transitions into the far-field pattern at the distance $z \gg a^2/\lambda_{dB}$, where the curvature of the wavefronts can already be approximated by plane waves [75]. The self-imaging (shifted² by half the period d) occurs at a specific distance, the Talbot length L_T :

$$L_T = \frac{d^2}{\lambda_{dB}} \quad (2.10)$$

The grating image continues unshifted at even integer multiples of L_T , and shifted by half a grating period d at odd integer multiples. At fractions of the Talbot length, self-images with fractions of the grating period are visible. The Talbot effect relies on sufficient spatial and spectral coherence from the source, which is usually not easily provided for typical molecular sources. A workaround is placing an additional grating G_1 with the same period $d_1 = d_2$ at the Talbot-length before the actual diffraction grating G_2 , an effect first described by Ernest Lau [79] for light. The slits of the first grating act as individual sources for wavelets coming from initially incoherent illumination. Coherence is generated by spatial confinement at each slit, however not between wavelets emerging from different slits, but instead each wavelet exhibits sufficient transverse coherence so that interference is possible when the wave is diffracted at the second grating. Individual slits create strong spatial confinement and therefore a high momentum uncertainty that causes large divergence so that each wavelet covers several slits at G_2 . The particle density of the interference pattern can be scanned with a third grating G_3 , again with the same period d_3 to provide spatial resolution. The (incoherent) sum of the individual particles then creates a density pattern, that differs significantly from the classically predicted Moiré effect. This interferometer configuration with three gratings $G_1 = G_2 = G_3$ separated by the distances $L_1 = L_2 = L_T$ is known as a homogeneous and symmetric Talbot-Lau interferometer. In-depth theoretical descriptions of this configuration can be found in [67, 80–82].

The grating period is determined by $d = \sqrt{L_T \lambda_{dB}}$ and scales therefore only with the square root $d \propto m^{-1/2}$. The scaling is more demanding in the far-field case: $d \leq \lambda_{dB} L/D \propto m^{-1} D^{-1}$, where L is the spacing of the interferometer taken to be the same length as L_T and D is the beam diameter [75]. Additionally, each slit of the first grating can be viewed as a source that illuminates the second grating, and the total signal throughput is enhanced by orders of magnitude in contrast to a single slit that prepares the necessary coherence in the far-field case. The slower scaling for increasing mass, the fact that one can work with initially incoherent sources, and the high transmission properties make the Talbot-Lau configuration suitable for realizing interferometry with massive molecules, and this type of interferometer is therefore our most promising candidate to perform interferometry with proteins.

2.3.2 Diffraction gratings

We discussed that diffraction can in principle occur at structures of arbitrary shape, however the two most prominent types for atom and molecule interferometry are material gratings and diffraction gratings made of light.

Material gratings

Various interference demonstrations utilized diffraction of particles on micro- and nanomechanical gratings. Advances in nanotechnology enabled the fabrication of nanostructures with arbitrary

²Unshifted self-images appear at the distance $L = 2d^2/\lambda_{dB}$. The definition used in Eq. 2.10 is more convenient for our purposes, though.

patterns, single/double slits [51, 83, 84], Fresnel zones [85], but most importantly diffraction gratings with equally spaced slits and periodicity d . Important examples include the first diffraction of Na-atoms on a gold grating with a period of $d = 0.2 \mu\text{m}$ in [14] and subsequent diffraction of sodium atoms on three SiN_x gratings, in a far field Mach Zehnder configuration [15]. The first diffraction of molecules on a SiN_x grating with $d = 200 \text{ nm}$ was conducted with helium dimers He_2 and trimers He_3 in [86]. An important step into the realm of complex molecules was the diffraction of large hot C_{60} -fullerenes on a SiN_x grating [16]. Overall, mechanical gratings offer a universal grating mechanism for atoms and molecules, particles that are not transmitted through the slits are absorbed and they offer arbitrary shapes that can be manufactured with grating periods smaller than (most) light wavelengths offered by laser systems. However, additional phase shifts due to dispersive effects from Van der Waals forces have to be taken into account for material gratings. The Van der Waals potential for atoms and molecules passing by transmission gratings has been modelled as follows [87]:

$$V = -\frac{C_3}{r^3} \quad (2.11)$$

where r is the distance from the surface. The material specific constant C_3 with the units $[C_3] = \text{eV nm}^3$ exhibited a linear dependence on the polarizability α for the atoms and molecule (He, Ne, Ar, Kr and D_2) diffracted at a 100 nm SiN_x grating in [66]. Polarizability increases for molecules, for example hydrogen has $\alpha_H \approx 0.58 \text{ \AA}^3 \times 4\pi\epsilon_0$ [88] where for C_{70} the polarizability is about 181 times stronger with $\alpha_{\text{C}_{70}} \approx 105 \text{ \AA}^3 \times 4\pi\epsilon_0$ [89]. The observed interference in the above mentioned C_{70} experiment [66] only agreed with the quantum prediction when the Van der Waals interaction was taken into account, with an obtained value of $C_3 = 0.09 \text{ eV nm}^3$ for the used gold gratings. The slower velocity of more massive molecules increases the interaction time and intensity of the effect [90]. Molecules can also stick to the grating and interact with subsequent molecules, or if large enough clog and even destroy the grating.

Optical gratings

Optical gratings can be used to circumvent these unwanted effects of mechanical gratings. They are realized by counter-propagating laser beams or a laser beam reflected into itself. The grating period is the spacing of the nodes or antinodes and is very precisely defined by half of the wavelength $d = \lambda/2$. Atoms, molecules, or nanoparticles interact with a light field where photon absorption can lead to photoionization, fluorescence, or intersystem crossing and phosphorescence. For molecules with a high degree of internal vibrational and rotational states, one can also observe vibrational relaxation and, depending on the photon energy, breaking of bonds leading to photodissociation or internal heating if the bonds cannot be broken. Molecules with a high electrical polarizability α receive an induced oscillating dipole moment from the field according to $\vec{d} = \alpha \vec{E}$, where α is a tensor in the general case. The electric field also induces a dipole potential in the second order [91]:

$$V^{\text{dip}}(x, y) = -\frac{1}{2} \alpha \overline{E^2(x, y, t)} = -\alpha \frac{I(x, y)}{2c\epsilon_0}, \quad (2.12)$$

with the time-averaged electric field and the local intensity $I(x, y)$. Equation 2.12 is valid for thin gratings in the Raman-Nath limit. One distinguishes between thin and thick gratings. For thick gratings, the propagation of the matter-wave through the spatial extension of the light wave in the propagation direction of the beam cannot be ignored. Two different regimes can be defined: if the grating is thinner than the Talbot length (Eq. 2.10), the Raman-Nath limit is satisfied, and for thicker gratings, Bragg scattering and channeling are dominating phenomena [38]. The imaginary part of the polarizability α quantifies absorption of photons, where the absorption cross-section

σ is given by [91] $\sigma = \text{Im}(\alpha)k_L/\epsilon_0$. The internal state of molecules is changed if one or multiple photons are absorbed. Random phase change by spontaneous emission is negligible since internal conversion is dominant for molecules, but phase change due to the transferred momentum $k\hbar$ by the photon has to be taken into account if the molecules are not removed from the detected signal. Internal relaxation processes take place on a shorter timescale than the absorption rate, so one can treat them as independent processes with a Poissonian distribution $p_{\bar{n}(x)}$, where the mean photon number $\bar{n}(x)$ is given by [91]:

$$\bar{n}(x) = \frac{\sigma}{\hbar\omega_L} \int I(x, z(t)) dt. \quad (2.13)$$

Both effects contributed to the successful quantum interference of (C_{60} , C_{70}) molecules in a far-field setup with one optical grating [91]. A Talbot-Lau configuration where the second grating is exchanged by an optical light grating has been discussed in [18, 67]. The interferometer was termed the *Kapitza-Dirac-Talbot-Lau* (KDTL) interferometer after the Kapitza-Dirac effect³ [92, 93]. Successful interference in the KDTL setup was realized for various molecules (C_{60} , C_{70} , $C_{60}F_{36}$, $C_{60}F_{48}$, and azobenzenes $C_{60}H_{12}F_{30}N_2O_4$) in [18, 67]. Further steps into higher mass regions were taken in [19] for TPP derivatives and perfluoroalkylated nanospheres with high masses up to 6910 Da. G_1 and G_3 are still mechanical in the KDTL, since the phases are incoherent at the first grating anyway, and the third grating is merely acting as an absorptive mask for detection, so Van der Waals interactions are not significant. Optical diffraction gratings are termed phase gratings if absorption is negligible due to off-resonant light waves and small absorption cross-sections.

Although Van der Waals forces are only relevant at the second grating, it can still be favorable to replace all gratings in a TLI by standing light waves. They have precise periods and cannot get damaged, but most importantly, pulsed lasers allow for pulsed TLIs that enable precise control of the interaction time, more on that in section 2.4. At least the first and the third grating need to act as absorptive masks to make that work. The grating must deplete the molecules from the molecular beam by transformation into a non-detectable state after photon absorption in the antinodes and allow free passage through the nodes. Molecules can effectively be removed from the beam by photoionization and subsequent deflection by an electric field. Another variant is photodissociation or fragmentation, where the photon breaks a bond in the molecule and removes it from the beam if the angle of separation is great enough. Both cases are examples of photodepletion gratings or measurement-induced gratings, since the processes can be viewed as a position measurement. The interference contrast is maximum for a TLI with three photodepletion gratings if single-photon absorption is sufficient for depletion, the contrast is reduced if one photon is not enough to deplete a molecule due to momentum change. High probability for depletion depends on high photon energy $\omega_L\hbar$, high absorption cross-section σ , and high photon density per area $N_P/A_L = E/(\hbar\omega_L A_L)$, where E is the laser energy in an area A_L . Pulsed laser beams can offer intensities in the TW regime, thus ensuring a high photon number per beam area, which is a necessary condition for gratings based on photon absorption. They additionally allow precise control of the interaction time of the molecular cloud with the laser field. Interferometry with pulsed beam splitters is generally referred to as interferometry in the *time domain*. In the next section, we will explore this concept more and combine it with an all-optical TL configuration to form a potential design for a protein interferometer.

³The original work was concerned with Bragg diffraction of electrons on an off-resonant light wave, but now the term is associated with diffraction on thin, off-resonant gratings in the Raman-Nath limit.

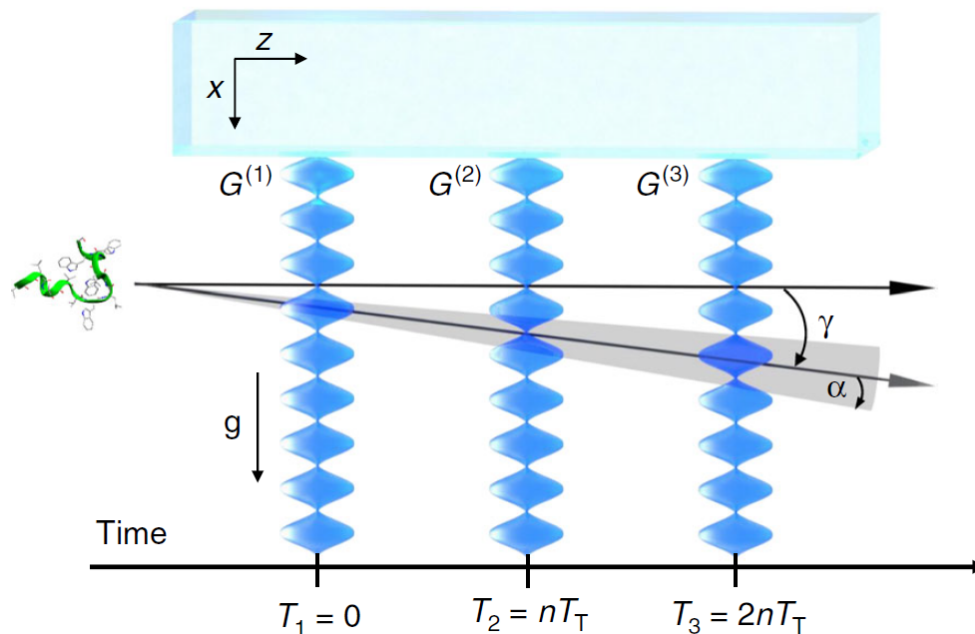


Figure 2.2: The pulsed Talbot-Lau interferometer concept of OTIMA: Three VUV laser pulses are reflected into themselves and create standing light waves that act as depletion gratings and exist for a precise amount of time. γ is the tilt of the molecular beam and α is diameter of the molecular beam. The coherent rephasing of the wave function occurs behind G_2 after multiples of the Talbot time nT_T . Figure extracted from [28].

2.4 Time domain interferometry

Pulsed light waves exist only for a precise amount of time. The advantage of this approach is that all particles will interact with the light grating for the same amount of time, regardless of their longitudinal velocity. This accurate timing is particularly suitable for high-precision measurements [38]. The first realization of a time-domain Talbot-Lau interferometer was published in 1997 [94]. More examples of interferometers with pulsed standing light wave gratings include [54–56].

OTIMA

A near-field Talbot-Lau interferometer with three laser pulses that act as absorptive gratings was proposed in [95], termed Optical Time-domain Ionizing Matter-wave Interferometer (OTIMA). We plan a protein interferometer based on this configuration, since it combines near-field efficiency, light gratings benefits, and time-domain precision. It is therefore worth describing the concept in more detail.

The OTIMA setup works in the time domain and was initially designed to use ionization as the main grating mechanism. Three short (≈ 8 ns) laser pulses with high photon energy in the VUV regime (F_2 -lasers at 157 nm, 7.9 eV, $E_P = 1$ -3 mJ) are retro-reflected at a single CaF_2 mirror surface. The single mirror design makes the setup more robust against vibrations and facilitates alignment of the three gratings. When particles pass through an antinode of the standing light wave, single-photon absorption can occur with high probability. The particle is ionized and deflected if the ionization threshold is within reach of the 7.9 eV photon energy. The standing light waves created during the laser pulse mimic the role of an absorptive mask analogous to the slits for a mechanical grating. G_1 prepares the necessary transverse coherence via optical ionization and acts purely as an absorptive mask. When the particle cloud is illuminated by the

second pulse, the transverse coherence of the wave function prepared by G_1 extends over several nodes and antinodes of G_2 . The optical dipole potential at G_2 additionally induces a phase modulation. The result is then an amplitude and phase-modulated molecular density pattern that is spatially probed by G_3 , again purely by an absorptive mask. The slit width of the grating can be fine-tuned with the pulse energy E_P , where higher pulse energy implies a higher probability for photon absorption further away from the antinodes and therefore reduces the opening fraction. Successful interference depends on the time delay between the pulses [95]. The characteristic quantity is the Talbot time T_t :

$$T_L = \frac{d^2}{\lambda_{dB}} = v \cdot T_t = \frac{mvd^2}{h} \Rightarrow T_t = \frac{md^2}{h}, \quad (2.14)$$

which is the point in time when self-imaging or phase revival of the grating occurs under coherent illumination. Equation 2.14 is independent of velocity and phase averaging effects caused by a finite velocity distribution, since all particles evolve for the same time duration ΔT_t during the pulses. However, this requires a specific velocity class for the particles to ensure they interact with all three gratings. A continuous source would require a uniform velocity of the particle beam, as in a non-uniform velocity distribution, particles would miss the laser pulse and reduce contrast in the interference pattern. This favors pulsed sources like laser desorption (LD) and MALDI or evaporative sources coupled to an Even-Lavie valve [96]. The latter was used in the first successful interference demonstration of anthracene (AC) clusters in an interferometer [97]. AC molecules were evaporated and injected into vacuum in a noble seed gas by an Even-Lavie valve with a duration of 30 μ s. The particles were cooled in the seed gas and formed clusters up to AC_{15} . The diffraction mechanism can naturally be extended to detection. Particles that have successfully passed through all three gratings are postionized by an additional laser pulse of 157 nm and detected by a TOF-MS with an MCP detector.

The propagation of molecules through the interferometer is treated in phase space with the Wigner function formalism as a transformation of the density matrix $\rho(x, x') = \langle x | \hat{\rho} | x' \rangle$:

$$w(x, p_x) = \frac{1}{2\pi\hbar} \int ds e^{ip_x s/\hbar} \langle x - s/2 | \hat{\rho} | x + s/2 \rangle. \quad (2.15)$$

A full treatment of the quantum model can be found in [28, 95]. The general evolution in the x-direction parallel to the grating vectors is governed by the free evolution Hamiltonian $H = p_x^2/2m$. Additional electromagnetic and gravitational force fields that cause acceleration a in the x-direction are incorporated, as well as a constant momentum p_γ , due to the transverse momentum of the molecular beam with a tilt angle γ . The evolution of the Wigner function after some time t is then:

$$w(x, p_x) = w \left(x - \frac{p_x t}{m} - \frac{p_\gamma t}{m} - \frac{at^2}{2}, p_x - p_\gamma - amt \right). \quad (2.16)$$

Transmission through the k^{th} grating $G^{(k)}$ is expressed by the transformation with the transmission function $t^{(k)}(x)$:

$$t^{(k)}(x) \rho(x, x') t^{(k)}(x)^*, \quad (2.17)$$

where $|t^{(k)}(x)|^2 \leq 1$ expresses the probability of no photon absorption in the grating. Integrating over the interaction potential (Eq. 2.13) for the optical grating with a flat-top profile yields the average number of absorbed photons n_0 and the accumulated phase shift ϕ_0 in the antinode:

$$n_0^{(k)} = \frac{4\sigma(\lambda_L)E^{(k)}\lambda_L}{hcA_L}, \quad \phi_0^{(k)} = \frac{16\pi^2 E^{(k)}\alpha_V(\lambda_L)}{hcA_L}. \quad (2.18)$$

Explicit evaluation of the transmission functions, the evolution of the Wigner function, and the expected visibility that take mirror imperfections and decoherence into account can be found in the above-mentioned references. The number n_0 in Eq. 2.18 indicates how many photons a passing molecule absorbs on average. For a single-photon process, $n_0 \geq 4$ guarantees an efficient depletion mechanism. Cross sections of $\sigma(157 \text{ nm}) = 1.1 \cdot 10^{-16} \text{ cm}^2$ were used in the above-mentioned AC cluster experiment to calculate the theoretically expected curves.

For a larger class of nanoparticles and especially proteins, ionization thresholds are generally too high to be reached by single-photon absorption. However, single-photon fragmentation can alternatively be used for labile bound clusters. In a subsequent experiment done in the OTIMA setup [47], interference of clusters of the biomolecules hexafluorobenzene (HFB) and vanillin was demonstrated by a combination of photoionization and photofragmentation. Both molecules have ionization energies above 8 eV, but they are thermally labile so that absorption of a single photon can break a cluster bond and lead to dissociation of the molecule. Fragmentation usually occurs without charge transfer, and neutral fragments cannot be deflected by an additional electric field; however, if cluster fragments have high enough off-axis velocities, they may miss the detection area of the detector. The authors report that fragments have off-axis velocities of 100 m/s and a forward velocity of 900 m/s (the angle is $\approx 100 \text{ mrad}$) and therefore miss the detector with an acceptance of 10 mrad. For HFB, photofragmentation was the dominant effect because the ionization energy lies at 9.97 eV. However, multiphoton ionization can also contribute to diffraction to some extent. Vanillin has an ionization energy of 8.30 eV, which is closer to 7.9 eV, and therefore a more probable process. It is additionally reported that especially for larger clusters, both effects compete, which is attributed to the fact that the ionization energy is lowered for higher mass clusters [47]. Successful interference was subsequently demonstrated in the setup for the naturally occurring polypeptide gramicidin A1, built of 15 amino acids with a total mass of $m = 1882 \text{ Da}$ [28]. The thermally labile polypeptide was launched with LD. The pulse repetition rate was 290 fs with a 343 nm wavelength and 70 μJ pulse energy, focused to a diameter of 100 μm to create intensities of the TW/cm^2 order. Sample efficiency was increased by a factor of 30 using fs instead of ns-desorption [28]. Different de Broglie wavelengths could be selected by changing the noble seeding gas from argon (600 m/s) to helium (1200 m/s). Gramicidin contains four strands of the amino acid tryptophan that can be ionized by photon energies of 7.9 eV, which was also exploited in the postionization scheme. The authors measured the number of ions as a function of the photon flux ϕ (see section 4.1.1, Eq. 4.2) to extract the ionization cross-section at a thermal average of 300 K to $\langle \sigma(\lambda_L) \rangle_{300\text{K}} \approx 4.7 \cdot 10^{-16} \text{ cm}^2$.

2.5 Charge control for a protein interferometer with photocleavable tags

If one wants to build on the OTIMA concept and reach beyond the mass of gramicidin to masses of 30-60 kDa, the scaling of the Talbot length T_L is an important issue. Equation 2.10 tells us that T_L scales linearly with mass m and velocity v , and quadratically with the grating period d^2 . The wavelength of 157 nm in OTIMA was chosen because the high photon energy of 7.9 eV offers the highest probability of photoionization for commercially available laser systems. However, ionization or fragmentation is further reduced for increasing molecular weight [98]. This represents a major challenge for the grating mechanism as well as for detection by postionization. The next important scaling factor is the particle velocity that has to be reduced for increasing mass. A MALDI source produces typical velocities in the range of 500-800 m/s (see section 3.1). If we assume a grating period of $d = 133 \text{ nm}$ (see below) and a velocity of about 300 m/s (seeded in Argon, 80 K), T_L for insulin ($m \approx 5.8 \text{ kDa}$) is $T_L = 7.7 \text{ cm}$, which implies a minimum

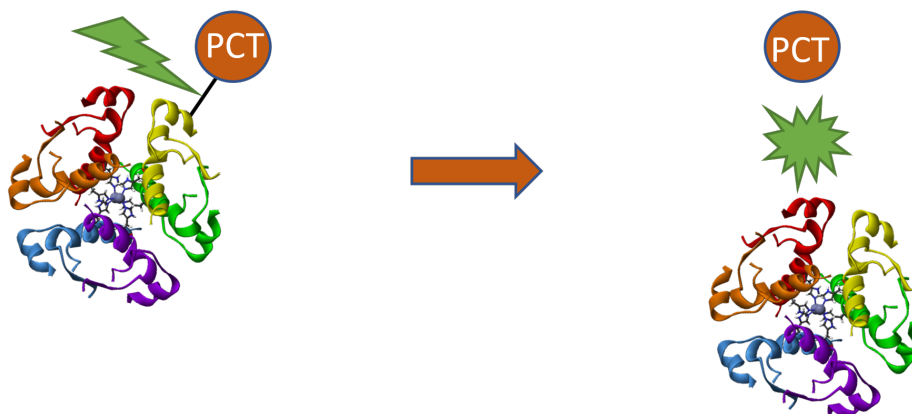


Figure 2.3: Illustration of the central principle of photocleavable tags (PCTs). The protein (here insulin) is tagged with an additional light-absorbing molecule that detaches at a precise spatial point upon irradiation with a specific wavelength. This enables charge control for neutralization and ionization or fragmentation.

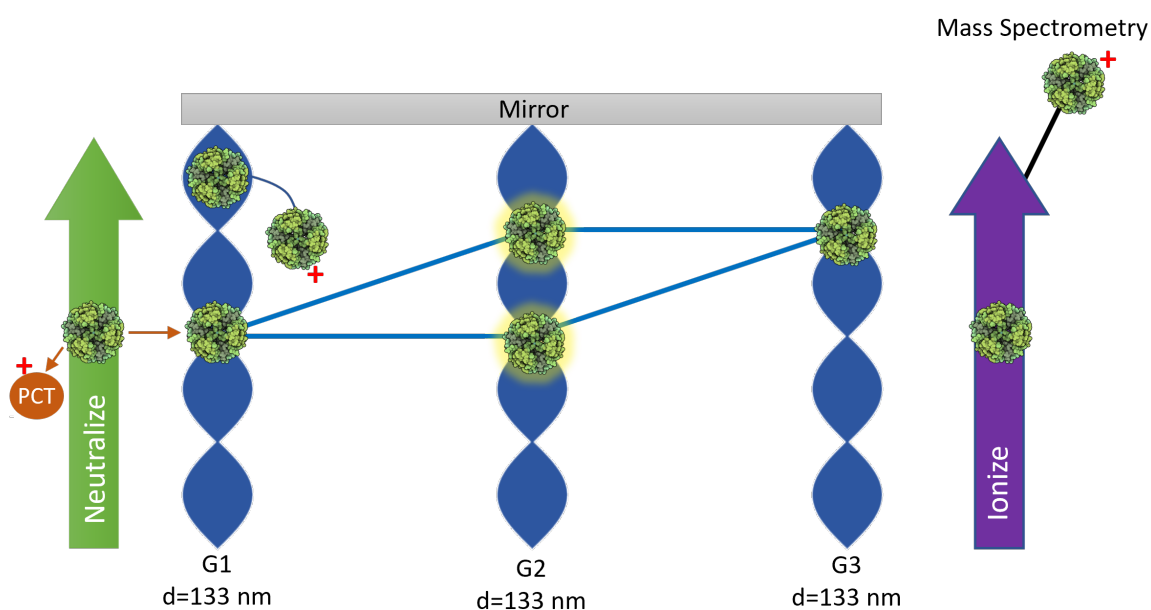


Figure 2.4: Concept of the three essential stages in the proposed protein interferometer with PCTs used for charge control. The charge necessary for neutralization is carried away by a PCT after irradiation of a laser pulse, for example at 532 nm. The diffraction mechanism is realized by either photoionization (shown) or photofragmentation (not shown), when a protein passes through the antinode of a 266 nm standing light wave. Ionization or fragmentation is enabled by a suitable PCT. Proteins that have successfully passed through three nodes are postionized by an additional laser pulse, the ionization is again triggered by a PCT.

interferometer length of 15.4 cm. A single mirror of this extension is already challenging to make and expensive, but still possible for high-quality fused silica optics that can be used for 266 nm. If we allow a mirror of approximately double the size (30 cm), Myoglobin ($m \approx 17$ kDa) would already need to be slowed to 200 m/s to keep T_L at 15 cm, and for more massive proteins with $m > 60$ kDa like hemoglobin, $v < 50$ m/s would be required. Such velocities are not in the range of LD and MALDI, see section 3.1. As mentioned above, LIAD would provide such velocities, however beam collimation, intensity and reproducibility are still open questions. Since MALDI produces mostly singly charged ions [99], slowing them down in the longitudinal direction with electric fields would be one solution. However, this approach introduces new difficulties. While it reduces the longitudinal component, it does not truly cool the system, and the previously insignificant transverse component now causes an increase in the divergence angle of the beam. A complex system of electrostatic einzel lenses could be a solution here. For the subsequent discussion, we want to assume a functioning slowing system. The question on our mind is how to neutralize the charged proteins before they enter the interferometer.

The problems of neutralization, ionization or fragmentation for diffraction, and postionization for detection can all be addressed with the technique of *photocleavable tags* (PCTs). We will only hint at the main idea here and how it can be used for interferometry; an in-depth discussion about PCT molecules and related experiments will be given in chapters 4 and 5. Figure 2.3 illustrates the main concept. The protein is chemically linked with a smaller molecule that contains photolabile protecting groups (PPGs) that detach from the protein when irradiated with a specific wavelength at a precise spatial location. This technique enables charge control; the PCTs can carry away a charge and neutralize the protein after cleavage, transfer a charge to the protein to ionize it, or simply cleave off without inducing a change in the charge state. Experiments with photocleavable tags have been conducted for 266 nm [100–103] and 532 nm [104], which will be discussed in detail in chapter 4, but so far they have not been used for interferometry. The wavelength of 266 nm is suited for a diffraction grating since it is still in the UV regime and only scales up the Talbot length T_L by a factor of about 3 compared to 157 nm. The wavelength is provided by the 4th harmonic of a Nd:YAG laser and usually offers higher pulse energies, as well as better coherence length. For example, a seeded Nd:YAG laser from InnoLas⁴ has a coherence length of about 25 cm, in contrast to only 1 cm provided by the F₂ lasers used in OTIMA. A longer coherence length improves the standing wave quality and spacing in the interferometer. A functioning protein interferometer requires at least two different types of PCTs, one for neutralization and one for ionization by 266 nm. If photofragmentation is used as the diffraction mechanism, a third tag is required for detection. It is desirable that the tags respond to clearly distinguishable wavelengths to separate the stages of neutralization and ionization or fragmentation. Charge reduction has been demonstrated for 532 nm with tags based on bodipys in [104] (see section 4.2.2). Employing this wavelength for neutralization would enable a clear separation between the neutralization and diffraction stages. The three essential stages are summarized in Figure 2.4.

⁴<https://innolas-laser.com/Products/Product-Overview.html?s=61D4D67F5EDA8B4E057C1465CFA5E8FE21A4210C>

Chapter 3

Beam generation

We already stated in chapter 2 that molecular beam creation based on effusive sources is not applicable to proteins. The source must vaporize proteins and should produce a strong ion signal that can be slowed with electric fields to keep the de Broglie wavelength on a feasible scale. The analysis of charged molecules from a sample is a central task in mass spectrometry and various techniques have emerged over the years that enabled the analysis of proteins. The most prominent examples include the already mentioned electron impact ionization (EI), *chemical ionization* (CI), *secondary mass spectrometry* (SIMS) methods like *fast atomic bombardment* (FAB) and *liquid secondary ion mass spectrometry* (LSIMS). We already mentioned electrospray ionization (ESI) and laser desorption/ionization (LDI), as well as Matrix-assisted laser desorption/ionization (MALDI) in chapter 2. EI and CI are methods to create ions in the gas phase for study, but are not useful to desorb intact proteins from a solid sample and can therefore be excluded. FAB enables desorption and ionisation of secondary ions from solids and liquids by a beam of high energetic neutral atoms. LSIMS uses ions to desorb analytes solved in a liquid matrix with insignificant conduction to avoid fragmentation. Both methods are considered soft, especially when the sample is embedded in a liquid matrix, and produce very similar spectra. They are therefore usually mentioned synonymously [99]. Charged or neutral beams of either noble gases (mostly Xenon [105], also Argon and Neon in FAB), Cs^+ ions in LSIMS) with high kinetic energies (4-8 keV (FAB), 5-25 keV (LSIMS) [99]) are focused on a target sample to generate a beam of secondary ions. Studies showed that one primary particle creates about 10^3 neutrals for about 0.02-1.15 ions [106], with velocities of the order 10^3 m/s [106, 107]. The usual area of application for FAB is in the range of $m/z = 3000$ [99]. Mass limits were reached for clusters of $[(\text{CSI})_n\text{Cs}]^+$ with $m/z = 90$ kDa [108], for proteins masses of $m = 23.463$ kDa have been detected [109]. Although a useful soft desorption ionization method, FAB has largely been replaced by the more powerful MALDI and ESI [99].

ESI and MALDI are the dominant methods to create charged beams of thermolabile biomolecules, that are subjected to mass analysis in a TOF-MS (section 3.5). Both techniques are applied in experiments by our group, ESI in the SuperMaMa project¹ and MALDI as well as pure laser desorption/ionisation without a matrix (LDI) in the experiments of chapter 5. They are used in different situations depending on the experimental requirements and goals and can be viewed as complementary [110]. ESI sends a liquid analyte solution through a small capillary to which a voltage is applied. The electric field between the capillary and a counter electrode creates a charged droplet that is sprayed into the gas phase. Free ions are created after evaporation of the solvent and are subjected to mass analysis. LDI on the other hand starts from a solid sample on a surface with the analyte embedded in a smaller, laser light absorbing matrix in the case of MALDI. Matrix and analyte are desorbed from the surface after irradiation by a laser pulse.

¹<https://www.supermama-project.eu/>

The matrix in MALDI facilitates evaporation and ionization of the analyte molecules without fragmentation, to make them available for detection. Ions are accelerated by strong electric fields of several kV in a TOF-MS, where they are separated by their m/z ratio (section 3.5). MALDI is the favorable option for our purposes, because in contrast to ESI, it predominantly produces singly charged ions which makes mass spectra clearer and easier to analyze [99]. It enables more precise control for slowing with electric fields since all particles receive the same force $\vec{F} = e\vec{E}$ if they carry only one charge. The challenge for neutralization by charge reduction is simplified in contrast to ESI, since the charge state only needs to change by one unit e . PCTs have been shown to be able to reduce the charge state in steps of one (see section 4.2). The pulsed nature of MALDI additionally makes it a natural choice for a pulsed protein interferometer.

More recent developments have achieved hybrid methods that are capable of separating the desorption stage from the ionization stage into two distinct events [110]. Particles are initially neutrally desorbed by fs laser pulses in the infrared (IR) regime and subsequently postionized by electrospray ionization, in a technique termed laser electrospray mass spectrometry (LEMS). A LEMS study has used a fs laser pulse (70 fs, 800 nm, 1.5 mJ pulse energy) to achieve an irradiance of 10^{13} W/cm² and successfully vaporize various biomolecules like the vitamin B₁₂ [111]. No matrix is needed since the IR-laser light nonresonantly couples to all the molecules investigated so far with this technique [110]. An electrospray beam with 3 μ L/min crosses the vaporized neutrals about 6.5 mm above the sample to ionize the neutrals, no ions could be detected in the absence of the ESI beam. Subsequent work followed that demonstrated the applicability of this technique to polypeptides [112] and proteins up to masses of ovalbumin (45 kDa) [113]. The absence of matrix can facilitate sample preparation and it is a pulsed process despite the fact that ESI is used for ionization. Charge distributions seem to be similar to ESI, the characteristic charge states for folded lysozyme (14.3 kDa) were centered around 10+ and 9+ in the LEMS spectrum for lysozyme [113].

For the light PCTs tested in chapter 5, it was sufficient to use LDI or MALDI. The following chapter will therefore present a more detailed description of MALDI and LD by diving further into proposed physical models behind these techniques, as well as explaining the different experimental steps applied in a typical mass spectrum measurement. We are concerned with UV-MALDI in the following, MALDI with infrared laserlight (IR-MALDI) is also a topic of research, but is not as widely applied as UV-MALDI [99] and it was not used for the experiments presented in chapter 5.

3.1 (Matrix assisted) laser desorption/ionisation

The idea to use pure LDI was already present in the 1960's [114] and used for mass analysis of small organic salts [115] and biomolecules [116]. The ionization of biomolecules with LDI proves to be exceedingly difficult for masses beyond 2000 Da [117]. LDI was therefore mostly ignored in favor of more successful methods such as FAB [99]. The addition of a laser light absorbing matrix soon revolutionized mass spectrometry and allowed the analysis of previously unattainable masses.

The term and general idea of MALDI was first coined by Hillenkamp, Bachmann and Karas in 1985 [118]. They irradiated a solid mixture of amino acids alanine and tryptophan with 266 nm laser light, where tryptophan acted as a laser light absorbing "matrix" to assist in successful ion formation of the non absorbing alanine. Tanaka developed a similar technique and was the first to apply the idea of embedding proteins in a matrix for mass spectrometry, reported m/z values reached up to $m/z = 100000$ [119]. Tanaka used a matrix of fine cobalt metal powder in glycerin, instead of the organic matrices now commonly associated with MALDI (section 3.2) and his work

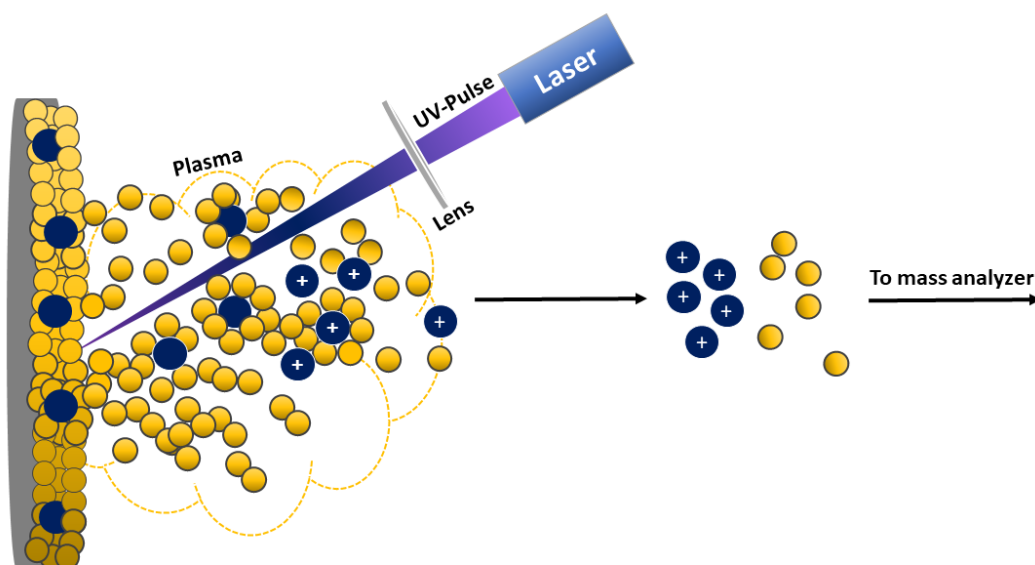


Figure 3.1: Schematic illustration of MALDI. Larger analyte molecules (blue dots) are embedded in a smaller UV-light absorbing matrix (orange dots). A focused UV-laser pulse vaporizes the sample and creates a hot gas plume. The plume expands adiabatically and subsequently cools. Ionization and desolvation takes place in the plume and the charged analyte can be extracted for mass analysis.

is now known sometimes referred to as soft laser desorption (SLD)² (not to be confused with pure laser desorption (LD) without any matrix). Subsequent work on the MALDI side ([120, 121]) resulted in successful desorption/ionization of proteins exceeding 67 kDa. MALDI is a standard analytical method in various fields today, just two examples are life sciences [122] and polymer research [123]. Despite this success, a consistent theoretical model with the ability to predict experimental outcomes like the ion yields to a satisfying accuracy is still absent to this day and knowledge about the MALDI processes is primarily gained from empirical studies, based on trial and error procedures. The exact processes responsible for ion formation are the main topic of controversy.

A typical MALDI process consists of several steps:

- (i) Sample preparation
- (ii) Laser desorption/ablation
- (iii) Ionization mechanism
- (iv) Ion extraction and spectrum analysis

We will take a closer look at each step in the following.

²Tanaka was awarded the noble prize for his work, however it is sometimes argued that the prize should have been bestowed to Hillenkamp and his colleagues, especially given that MALDI is now the established technique in the field. On the other hand, Tanaka was the first to apply the concept to proteins.

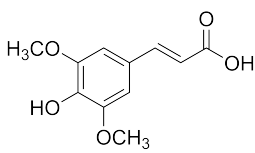
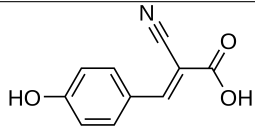
IUPAC name	mass, ionization potential	solvent	structure
3,5-Dimethoxy-4-hydroxycinnamic acid	$m=224.21$ Da, IP=8.67 eV	purified water, acetone, acetonitrile, chloroform, dimethylsulfoxide (DMSO)	
α -Cyano-4-hydroxycinnamic acid	$m=189.170$ Da, IP=8.5 eV	acetonitrile, water, ethanol, acetone, DMSO	

Table 3.1: MALDI matrices used in experiments.

3.2 Sample preparation

The first step in preparing a sample for MALDI is the choice of a suitable matrix. A list of common UV-absorbing matrices and their properties can for example be found here [124]. For the experiments described in chapter 5, sinapinic acid (SA) and α -Cyano-4-hydroxycinnamic acid (CHCA) were used. Some properties and the structure are depicted in table 3.1. MALDI matrices have low molecular weight (100-300 Da) to be volatilizable and easy to distinguish from the analyte in the mass spectrum. Additionally, they have high absorbance in the UV regime to absorb at typical laser wavelengths 337 nm (N_2) and 355 nm (3^{rd} Nd:YAG harmonic). It is also important that the matrices do not have too strong vapor pressures ($3.12 \cdot 10^{-7}$ Torr at 25 °C for SA) and not evaporate during preparation, but not low enough to not evaporate at all. Furthermore, MALDI matrices are usually acidic to act as proton donors.

The solutions for analyte and matrix are then diluted to achieve the wanted matrix/analyte molecule ratio R , which can cover a wide range from $R = 10^7$ to even $R = 10$. The effect of the matrix is reduced for larger analyte concentrations and the spectra are more similar to LDI [99]. Matrix and analyte are then pipette spotted on targets like stainless steel plates to co-crystallize during solvent evaporation. This is a crucial process and a variety of different preparation techniques is reported in literature [125]. A standard preparation method is the so called *dried droplet* (DD) technique, where a purified analyte solution is spotted onto the target plate until the solvent has evaporated (usually in air) and is then overlayed with 1 μ L of appropriate matrix solution. Drawbacks of this method are hot spots of high intensity caused by uneven crystallization and poor spectra quality for low concentrated samples, because molecules dilute across larger areas. Different variations of this method exist, for example *rapid crystallization*, where sample and matrix are spotted one after another and let dry in a vacuum chamber, or the *sandwich method*, where a layer of matrix/analyte/matrix is prepared, by letting each layer dry before the next one. Another interesting possibility is to use an electrospray source for deposition on the target plate [126]. The authors used an ESI-source to create spots of varying thickness on a plate, reporting strong ion signals for all layers, as well as 2 times higher absolute and relative signal stability for thin layers compared to the DD method, due to more homogeneous sample distribution.

Direct mixing was the preferred preparation method for the experiments described in chapter 5. Analyte and matrix solutions are mixed together beforehand to ensure the desired ratio of matrix to analyte on the target. Subsequently, the sample is put into a vacuum chamber for rapid crystallization. The advantage of this method is that the analyte is more incorporated in the matrix, which results in higher signal intensity and faster crystallization. There is no universal

procedure in finding the right matrix, solvents, particle ratios and preparation method for obtaining clean mass spectra in a MALDI measurement and it is therefore mostly a process of trial and error in the lab.

3.3 Laser desorption/ablation

Crucial laser parameters are wavelength, pulse duration and energy, photon energy, repetition rate and pulse shape. Typical laser systems in UV MALDI are:

- N₂-lasers, 337 nm, 3-5 ns, 30-60 Hz, 3.68 eV
- 3rd-harmonic of Nd:YAG lasers, 355 nm, 4-7ns, 3.49 eV, 20 – 5000 Hz
- 3rd-harmonic of Yb:YAG lasers, 343 nm, few 100 fs, 3.61 eV, 50 - 500 kHz, (fundamental at 1030 nm can be used in IR-MALDI)

Ionization thresholds are in the 3-photon range for these laser systems (table 3.1). N₂-lasers are relatively cheap and frequently used in MALDI experiments, however the limited repetition rate of 50-60 Hz and an average shot lifetime of 10⁷ limits their capabilities. Higher repetition rates can be achieved with the solid state laser systems listed above, but they usually suffer from smaller spot sizes, which can be circumvented by beam modulators [99]. A common threshold value for the generation of ions is the power P per area A or *threshold irradiance* of $P/A = 10^6$ W/cm² [127, 128]. Below this threshold no ions are formed, for higher values ionization can grow rapidly with the 5th to 9th power of the irradiance. The highest ion yield strongly depends on the matrix:analyte ration, studies for Cytochrom C have for example determined a ideal ratio of 4000:1 [129]. About double the laser power was needed if analyte concentrations were too high or too low. In the case of higher concentration, the effect of the matrix is reduced and more energy is required to achieve high enough absorption rates by the matrix. Conversely, in the case of less analyte concentration more material has to be vaporized to produce analyte ions. Studies to determine the number of created ions and neutral were conducted in [130]. Desorbed neutrals were collected on a quartz crystal microbalance, with a ration of ions to neutrals of 10⁻⁵. Other studies have shown that neutrals start to be evaporated at a minimum *laser fluence* ($F = E/A$) of around $F = 11$ mJ/cm² [131], where E is the pulse energy.

MALDI spectra with high intensity and low fragmentation are usually achieved for values in the range of the thresholds from above, laser spot sizes of 100-200 μm are favorable because the cover several μm sized crystals and can therefore average out different orientations of the crystals to the laser orientation [99]. The typical laser systems used set the time scale for desorption to ns, however ps and fs pulses have been investigated as well [132, 133]. The light is absorbed by the matrix electrons that store the energy and act as a buffer. After absorption, the energy is converted into heat via non radiative relaxation in the matrix molecules. The time scale for dissipation is limited to ns because lifetimes for most commonly excited singlet states S₁ of matrix molecules are of a few hundred ps up to ≈ 1 ns, before decay by intermolecular relaxation [134]. This creates a limit for the heat pulse duration that results from excited state decay, even in the case of ps or fs laser pulse durations. The transition from solid state to an expanding, dilute gas plume afterwards is of the order of μs [124]. It is consensus today that this difference in time scales divides ion generation in MALDI into two parts, the generation of ions in primary and secondary processes. Primary processes occur on the ns time scale, during or shortly after the laser pulse. Examples of primary processes in matrix molecules include photoexcitation and multiphoton ionization, thermal ionization and charge separation. Different processes may be favoured depending on the proposed model for primary ionization. We will briefly discuss the

two most important ones in section 3.4. In contrast, secondary processes occur in the gas plume during expansion and involve processes like proton transfer, recombination and electron transfer, among others.

MALDI fluxes are correlated with the created vapor pressure during heat dissipation. Evidence for this is observed dependence of the threshold fluence (F_T) required for a desorption signal on initial sample temperature [135]. It is now common to distinguish two different regimes for the transition of the sample into the gas phase, *desorption* and *ablation* [124]. For the desorption regime, small parts of the sample are smoothly evaporated into the gas phase, containing no larger chunks of material like clusters or mesoscopic particles. The transition into the ablation regime is reached at the critical fluence F_c [136]. This threshold characterizes the transition from smooth phase change to a sudden phase 'explosion'. This sudden ejection of material can lead to nucleation processes where clusters and larger bulks of material are formed. Determining F_c theoretically is a difficult task, because it strongly depends on the experimental conditions [136]. Molecular dynamics simulations (MDS) have shown that for typical MALDI fluence values in the range 1-9 mJ/cm², both regimes are theoretically possible, under specific experimental conditions of laser pulses between 15-150 ps at a wavelength of 337 nm [137]. Knochenmuss has pointed out that the transition between the two regimes can be modelled as a function of energy density, when operating at moderate higher fluence values in the range 5-50 mJ/cm² [138].

The expansion after laser desorption/ablation can be treated as an adiabatic jet expansion [139], similar to the supersonic beams mentioned in section 2.1. The initial dense plume expands until the pressure of the gas reaches the background pressure and cools down during the process. Experiments report quite distinct values for initial plume temperatures from 500-1000 K [140, 141]. Molecular dynamics simulations came to similar conclusions [142]. The cooling process slows down with increasing expansion of the gas. Additionally, the temperature in the plume seems to be subject to a gradient, rather than uniformly distributed [124]. The gas plume predominately expands into the axial direction, typically reported velocity distribution peak around 500-800 m/s and can extend past a 1000 m/s [143], see experiments of chapter 5.

3.4 Ionization mechanisms

Theoretical models capable of predicting MALDI mass spectra with satisfactory accuracy and in line with empirical data are still not fully developed. The generation of the detected ions remains the most controversial part. However, the division into primary and secondary processes described above is scientific consensus. While secondary models converge, there are a variety of different models for primary ion generation. Primary models focus on ions generated during or shortly after the laser pulse. The role of the matrix is either extended or reduced depending on the model. The following section will briefly discuss the two most prominent candidates of primary ionization models, the cluster ionization model mostly developed by Karas et. al. [144, 145] and the photochemical model of Knochenmuss [124].

3.4.1 Primary ionization: Cluster model

The cluster model diminishes the role of the matrix solely to a necessary tool for the molecules to be lifted into the gas phase, instead of being inherently involved in ion formation. Analytes are assumed to already exist in a charged state in solution and this model states that they maintain the charge state also in the dried solid. Higher peptide ion yields are correlated with a reduced pH-value in the matrix/analyte solution, which indicates preformed $[A+H]^+$ or $[A+alkali]^+$, where

A is the analyte [99]. The critical process is the separation of ions from counterions (charge separation). These ions are formed and contained in hot clusters that are produced during laser ablation. Analyte ions can already possess net positive or negative charge in the cluster and can subsequently become free ions by desolvation from the cluster. Recombination can take place to a large degree, however unevenly. The final observed ion yield is therefore a stochastic process. For this reason, the model was initially known as the 'lucky survivors model' [144]. A variety of (secondary) processes can additionally take place inside the clusters after ablation. Preformed matrix ions can transfer their charge to an analyte molecule (intracluster charge transfer), multiple charged analyte ions can undergo neutralization with electrons and leave singly charged ions in the cluster that can be desolved. Different routes of desolvation have also been suggested, distinguishing between 'hard' and 'soft' desolvation [146]. In soft desolvation, neutral matrix is evaporated from the cluster, leaving potential high charged states unchanged, whereas 'hard' desolvation creates charged matrix ions that leave only low to singly charged analyte ions.

It was pointed out that this model cannot fully account for characteristic MALDI phenomena. The abundance of clusters in small fluence regimes makes it hard to explain why ions can still be encountered in this regime and the fact that the model assumes ions in a preformed state makes it hard to explain effects such as sweet spots and the *matrix suppression effect* (MSE, section 3.4.3) [147].

3.4.2 Primary ionization: Photochemical model

In contrast to the model discussed above, the photochemical model enhances the role of the matrix to be crucially involved in ion generation. The model was mostly developed by Knochenmuss [124] and we follow his description here. It assumes ionization of matrix molecules during the laser pulse and subsequent ionization of analyte molecules in the gas plume by secondary processes. Since electron excitation energies for matrices like CHCA and SA all lay in the 3-photon range for commonly used lasers, direct photoionization of matrix molecules will only play a minor role in the process. The proposed main mechanism for ion generation is a combination of energy concentration and migration inside the solid. Energy can be concentrated and redistributed in events termed 'pooling'. A pooling process can be illustrated as follows: matrix electrons can populate the first excited singlet state S_1 by photoexcitation. If two molecules in excited singlet states are close enough so that their wave functions can overlap and interact, redistribution of energy can occur, leaving one molecule in a higher excited state and the other one in the ground state: $(S_1, S_1) \rightarrow (S_0, S_n)$. In addition, excitations can migrate inside the solid crystal, a process that is described by movement of pseudo particles referred to as excitons. This process depends on intermolecular wave function overlap similar to pooling, however now between a singlet ground state S_0 and excited state S_1 . Both pooling and exciton migration were already well known in solid-state aromatics [148]. For the common MALDI matrix Dihydroxybenzoic acid (DHB), excitons move on a time scale of around 50 psec, making large migration possible in the excited state lifetime of around 1.5 ns. This exciton 'hopping' can enable multiple pooling events, ultimately resulting in ionization of a matrix molecule: $(S_n, S_1) \rightarrow (M^+, S_0)$, where M^+ is a positively (radical) matrix ion. For DHB, these processes have been quantitatively modelled into a set of coupled differential equations, being able successfully predict certain matrix only phenomena, however effects like sweet spots can still not be explained by this model [147].

3.4.3 Secondary processes

Possible processes that happen in the gas plume include neutralization due to recombination of ions and counterions and various types of charge transfer due to collisions of matrix and analyte

particles with each other, neutral or charged, although reactions with neutrals are probably the usual case because the number of neutrals exceeds the number of ions as discussed above. Matrix ion pairs can for example be created via auto ionization by partly photoinduced and thermal processes, it takes around 7.5 eV to create a matrix ion pair of radical cation and anion matrices in DHB [$2M \rightarrow M^{\cdot+} + M^{\cdot-}$]. At this point, it is useful to distinguish the different types of matrix ions that can be encountered and the appropriate terminology. Matrix ions with an additional or absent electron are referred to as a radical cation $[M^{\cdot+}]$ or anion $[M^{\cdot-}]$. In the case of protons they are referred to as protonated $[M-H^+]$ or deprotonated $[(M-H)^-]$ ions. Matrix molecules with larger charged adducts are called cationized matrix molecules, for example: $[M-Na^+]$, $[M-K^+]$. Sometimes also more exotic species can be encountered like $[M-CO_2]$ or $[M-H_2O]$ or even $[M+2H^+]$. Under typical plume conditions these species can also interconvert [124].

Matrix ions can react with analyte ions in different ways. For proteins and peptides, the most common reaction is proton transfer $[M-H^+ + A \rightarrow M + AH^+]$, where A refers to a general analyte molecule. This reaction is preferred because of the very high proton affinities of amino acids between 885-1025 kJ/mol. Other possible reactions include electron transfer from analyte to matrix $[M^{\cdot+} + A \rightarrow M + A^{\cdot+}]$ or cationization $[M-Na^+ + A \rightarrow M + A-Na^+]$. An interesting consequence of secondary plume reactions is the matrix suppression effect (MSE). As the name suggests, under specific conditions matrix ions can be completely absent in the mass spectrum. This is a consequence of secondary reactions, analyte ions can completely deplete the matrix ions, for example by deprotonation. Different matrix ions can additionally interconvert into protonated matrix ions and can again be deprotonated by analyte particles, which results in suppression of the matrix signal. It is frequently pointed out that the MSE cannot be explained by the cluster model, since intracluster charge transfer of matrix to analyte would always require more analyte than matrix molecules present in the clusters, a statistical impossibility. Analogous, analyte suppression is possible as well in the case of multiple analytes. Conceptually, one analyte B can dominate reactions with the matrix, or ions of analyte A can subsequently be depleted by analyte B, resulting in suppression of A [149].

3.5 Ion extraction

The natural choice to extract pulsed ions in MALDI are pulsed time of flight mass spectrometers (TOF-MS)³. TOF-MS apply a fixed amount of kinetic energy to charged particles of different mass by acceleration in a uniform electric potential U . Since $v = \sqrt{2E_{kin}/m}$, heavier particles will move slower than lighter ones when they receive the same kinetic energy. The transferred kinetic energy is equal to:

$$\frac{mv^2}{2} = qU \quad (3.1)$$

According to Eq. 3.1, particles with higher charge states $q = ze$ receive more kinetic energy and will therefore show up at earlier timings in the spectrum. The particles move across a fixed distance during an amount of time $\Delta x = v \cdot t$. The mass to charge ratio m/z can be extracted:

$$t = \sqrt{\frac{m}{2qU}} \cdot \Delta x \quad (3.2)$$

$$\rightarrow \frac{m}{z} = 2eU \cdot \left(\frac{t}{\Delta x}\right)^2 \quad (3.3)$$

³For that reason, one regularly encounters the term MALDI-TOF

The mass to charge ratio m/z is sometimes encountered with units of mass and charge, however it is usually defined as a dimensionless quantity in mass spectrometry [99]. A linear TOF is suitable in the simplest case for ions generated in MALDI or LDI. Ions are *generated* by MALDI or LDI between two plates at high voltage and ground potential. The potential accelerates the particles according to Eq. 3.1 (Note that depending on the applied voltage, the plates can extract only positive or negative ions. Some TOFs have the ability to switch between positive and negative extraction). After the acceleration stage, the ions move on a free drift path to a suitable detector, usually a multichannel plate (MCP) that generates secondary amplified electron currents. The volt signal of the current vs time is displayed on an oscilloscope or on a computer via a fast analog-to-digital converter [99]. The resolution can be reduced by the following effects, if the ions are created in a pulsed fashion but extracted by a continuous voltage: ions have different starting energies, start at different times or positions, or have different directions of motion. All are common problems if the ions are created in the extraction zone and extracted continuously. A way to improve time resolution is achieved with a reflectron TOF. After the drift region, particles enter a region by a compensating electric field, which in practice can consist of a system of ring electrodes with increasing potential. Ions penetrate into the reflector until they have lost their kinetic energy and are reflected at an angle. As ions with higher energy penetrate deeper, different starting energies can be partially compensated, increasing the resolution. A focusing effect occurs as well [99].

An additional improvement for ion extraction is the use of an orthogonal TOF. In this case, the ions are extracted by voltage pulses orthogonal to the direction of motion of the molecular beam. This has the advantage that the kinetic energies of the particles are no longer as high as with direct generation in the ion source, usually in the range of 10-20 eV [99]. The two-stage extraction introduced by Wiley and McLaren [150] is usually used. There are two acceleration stages in this configuration, which allows for finer tuning of the parameters and can correct for different starting positions, increasing the resolution. The orthogonal extraction is the method applied in the TOF used in our experiments (section 5.3).

Chapter 4

Photoionization and Photocleavables

We have already illustrated the idea of photocleavable tags (PCTs) in chapter 2. This chapter further explores the topic and discusses specific experiments with PCTs. In addition, potential PCT candidates are presented that have been experimented with in chapter 5. The efficiency of photocleavable tags (PCTs) is characterized by their absorption behavior, specifically, whether the cleavage process is initiated by single or multiphoton absorption. This process is mathematically analogous to single and multiphoton absorption/ionization. We will therefore discuss the topics of single and two-photon absorption in the following section. Several experiments performed for PCTs in Vienna are discussed in section 4.2.

4.1 Photon absorption/ionization

The excitation of an electron beyond the ionization threshold can occur in different ways. The molecule can off resonantly absorb a single or multiple photons with enough energy to ionize the particle without exciting it into a physical intermediate state. This case is referred to as off resonant single and multi-photon ionization, respectively [151]. If however the electron is excited into one or several intermediate states during photon absorption, the process is called *resonant enhanced multi photon ionization* (REMPI). This process can be thought of as an electron climbing up a ladder of excited states upon each absorbed photon, until it escapes the bond state. The intermediate states need long enough life times to not decay before a subsequent photon can be absorbed [152]. We will confine the discussion to one- and two-photon ionization, as these are relatively straightforward to address and multiphoton processes are not desirable for an efficient grating mechanism.

4.1.1 Single photon absorption/ionization

Single photon absorption occurs when the photon energy is high enough to excite an electron beyond the ionization threshold of stable bound states into a continuum [152]. The number of ionized particles per time n_i after single photon absorption is equal to the rate of change of (electronic) ground state particles in the laser beam area per time:

$$n_i = -\frac{dN_g(t)}{dt} = \sigma_1 \phi N_0 \quad (4.1)$$

with the total number of molecules in the laser beam area $N_g(t = 0) = N_0$, the single photon absorption cross section σ_1 with $[\sigma_1] = \text{cm}^2$ and the photon flux (number of photons per area and time) $\phi = I/h\nu$, where I is the laser intensity and $h\nu$ the photon energy. The differential equation can be solved to obtain the number of particles in the ground state after the laser pulse

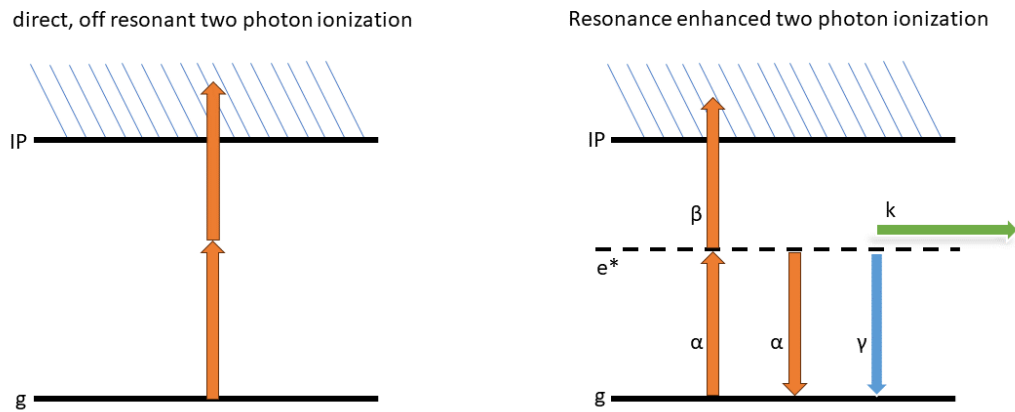


Figure 4.1: Energy level schemes for off resonant (left) and resonance enhanced two photon ionization (right) for photons with equal energy. The sum of the photon energies surpasses the ionization potential (IP) of the electron in the ground state g . In the second case, the electron is first excited into an excited state e^* with the absorption rate α . β is the rate for the second ionization step, k is the rate for non radiative relaxation in molecules, γ is the spontaneous emission rate and α is also the rate for stimulated emission. The rates α and β can be extended to multi-photon processes as well.

duration τ :

$$\begin{aligned} \frac{dN_g(t)}{dt} &= -\sigma_1 \phi N_0 \\ \int_{N_g(0)}^{N_g(\tau)} \frac{dN_g(t)}{N_0} &= -\int_0^\tau \sigma_1 \phi dt \\ \Rightarrow N_g(\tau) &= N_0 e^{-\sigma_1 \phi \tau} \end{aligned}$$

The number of ionized particles $N_i(\tau)$ after the laser pulse τ is then:

$$N_i(\tau) = N_0 - N_g(\tau) = N_0(1 - e^{-\sigma_1 \phi \tau}) \quad (4.2)$$

We can rewrite the exponent of Eq. 4.2 in terms of photons per area:

$$\phi \tau = \frac{E_L}{h\nu A_L} = N_P/A_L = \Phi \quad (4.3)$$

with the laser pulse energy $E_L = I \cdot \tau$ and the total number of photons per laser beam area N_P/A_L . Φ is also sometimes referred to as the photon flux, we will call it photon fluence.

4.1.2 Two photon absorption/ionization

When photon energies are not sufficient for single photon ionization, two photon ionization is still possible if the photon density in the laser beam is high enough and the ionization threshold

is in reach. Two cases of two photon ionization can be distinguished: off resonant two photon ionization and resonance enhanced two photon ionization [151], see figure 4.1. In the off resonant case there is no excitation to an intermediate energy state and the sum of two absorbed photons is enough to directly excite an electron beyond the ionization threshold. Another point of view is that the first photon excites the system to an off resonant *virtual state* with energy detuning $\Delta E = \hbar\Delta\omega$. The lifetime of this state is very short ($\approx 10^{-15}$ s [153]) and of the order $\Delta\omega^{-1}$ [154]. The probability for off resonant two photon absorption during a laser pulse can be estimated with lowest order perturbation theory to [153]:

$$\frac{dP}{dt}(t) = (1 - P(t))\sigma_2\phi^2(t) \quad (4.4)$$

where σ_2 is the generalized two photon absorption cross section with units $[\sigma_2] = \text{cm}^4 \text{ s}$ and $\phi = I/\hbar\omega$ the photon flux. For the special case of an intensity independent cross section, one can derive an exponential law for the ion yield after the laser pulse [153]:

$$N_i(\tau) = N_0 - N_g(\tau) = N_0(1 - e^{-\sigma_2\phi^2\tau}) \quad (4.5)$$

The case of two photon REMPI is more complicated since different competing processes are possible during the lifetime of the excited state. The processes are illustrated in figure 4.1. The rate of absorption is α , subsequent ionization happens with the rate β and k is the nonradiative rate for relaxation in molecules. Stimulated emission depopulates the excited state with the same rate as for absorption α and spontaneous emission can additionally occur with the rate γ . All rates have dimension s^{-1} . Absorption and ionization are separated and two different cross sections are assigned to the respective processes. In the general case, absorption and ionization can be multi photon processes themselves. One speaks of a $(n + m)$ REMPI process if n absorbed photons lead to an excited state and m absorbed photons cause ionization. Several intermediate excited states are not considered here. Different cross sections are assigned to absorption and ionization, in the case of an $(n + m)$ REMPI process [155]:

$$\alpha = \sigma_n I^n \quad (4.6)$$

$$\beta = \sigma_m I^m \quad (4.7)$$

where $[\sigma_n] = \text{cm}^{2n} \text{ s}^{n-1}$. For a (1+1) REMPI we get $\alpha = \sigma(1)I$ and $\beta = \sigma(2)I$, with the cross section for excitation $\sigma(1)$ and ionization $\sigma(2)$. The processes can be modelled into a system of coupled rate equations [155]:

$$\frac{dN_0}{dt} = -\alpha N_0 + (\alpha + \gamma)N^* \quad (4.8)$$

$$\frac{dN^*}{dt} = \alpha N_0 - (\alpha + \beta + \gamma + k)N^* \quad (4.9)$$

$$\frac{dN_i}{dt} = \beta N^* \quad (4.10)$$

Where N_0 , N^* , N_i are the number densities (molecules/ cm^3) for molecules in the ground state, excited state and ionized, respectively. The general solution is quite complicated, if we neglect nonradiative relaxation, one can approximate two different cases. Eq. 4.5 is encountered again in the case of a high decay rate or short lifetime of the virtual state $\gamma = \tau_i^{-1}$ ($\gamma\tau \gg 1$, $\gamma \gg \sigma(1)\phi$ and $\gamma \gg \sigma(2)\phi$) [153]. The generalized two photon cross section of Eq. 4.5 is expressed by

$\sigma_2 = \sigma(1)\tau_i\sigma(2)$ [154], where τ_i is the lifetime of the short lived virtual state. For the case where the laser pulse duration dominates the decay rate ($\gamma\tau \rightarrow 0$) one finds [153]:

$$N_i/N_0 = 1 + \frac{e^{-(s_1+s_2)\phi\tau/2}(s_1 - s_2)}{2s_2} - \frac{e^{-(s_1-s_2)\phi\tau/2}(s_1 + s_2)}{2s_2} \quad (4.11)$$

with $s_1 = 2\sigma(1) + \sigma(2)$ and $s_2 = \sqrt{4\sigma(1)^2 + \sigma(2)^2}$. In this case the generalized 2 photon cross section is expressed with half the temporal width of the laser pulse duration $\sigma(1)\frac{\tau}{2}\sigma(2)$ [153].

4.2 Photocleavable tags

Photocleavable tags, sometimes also called photocages or photoremovable protecting groups (PPGs) enable the release of various chemicals at a precise spatial and temporal location after photon absorption. They play an important role in various biological and chemical processes and a large amount of different PPGs have been studied and characterized in solution [156]. The goal of our group is to utilize them as a tool for matter-wave interferometry and therefore we need to characterize their photoresponse in vacuum. PCTs contain chromophores that absorb photons and produce an excited state. This excitation can facilitate the breaking of certain bonds in the PCT. The breaking or cleavage of these bonds can alter the charge state or other properties of the parent molecule.

Two important processes for charge state control are *heterolytic cleavage* and *homolytic cleavage*. Heterolytic cleavage is a process where a covalent bond breaks and both electrons are transferred to one bond partner, the result is charge separation and the creation of a cation-anion pair, if an initial neutral bond is broken. This process can for example be utilized to ionize the parent molecule after photon absorption. Homolytic cleavage describes the opposite case and the bond is broken by splitting the two electrons equally between the bonding partners with no charge transfer to create free radicals. This can for example be applied to neutralization when the tag is initially negatively charged (see below). A third important process is the charge neutral *H-transfer*. The central task in characterizing a potential tag is to interpret the mass spectrum after laser irradiation to deduce the nature of the cleavage process and the efficiency, that is if a single, two or multiphoton process triggers the cleavage. If for example the cleavage mechanism results in postionization of the parent molecule, one can plot the ion yield as a function of pulse energy and fit functions Eq. 4.2 and Eq. 4.4 to determine the process and the photon absorption cross section. Photocleavage experiments that demonstrated charge reduction have been carried out by our group. The next section discusses experiments that used nitrobenzylethers as PPGs for 266 nm to demonstrate charge reduction of peptides and the neutralization of insulin in the gas phase. Recent experiments have demonstrated successful uncaging of large peptides with PPGs based on bodipy with a pyrridinium linker using green light (532 nm). Finally, we introduce test particles based on porphyrine and phthalocyanine that were experimentally investigated in this thesis.

4.2.1 Nitrobenzylethers

A proof of principle investigation was conducted in 2017 [100], by our group in collaboration with chemists from university of Basel¹. A PCT based on the well studied UV-photosensitive nitrobenzyl group [156, 157] was designed and tested for photocleavage with molecules both in solution and isolated in the gas phase. The tag consisted of a 2-(phenoxymethyl)-1-nitrobenzene

¹<https://koehler.chemie.unibas.ch/en/>

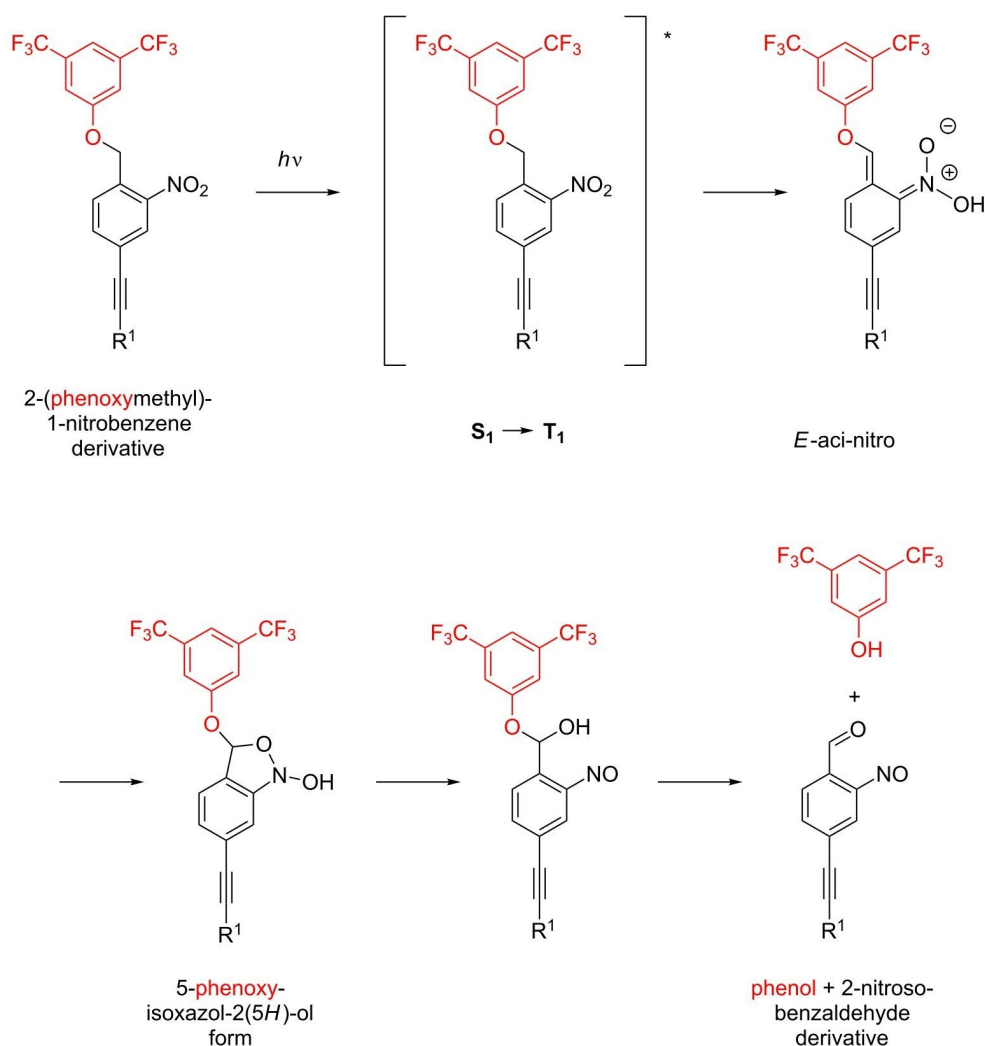


Figure 4.2: Illustration of the cleavage mechanism. When a UV-photon is absorbed, the tag undergoes a cleavage reaction that dissociates the phenol subgroup from the rest. Figure adapted from [100].

derivative with an additional ethynyl subgroup that enables connection to different molecules (R^1) and additional CF_3 groups attached to the phenyl subgroup. The structure and cleavage mechanism is illustrated in figure 4.2. The compound is excited into a singlet state S_1 after absorption of a 266 nm photon and relaxes to a triplet state T_1 afterwards. A subsequent chemical reaction leads to cleavage of the phenol group. The tags were attached to a benzene ring (at position R^1 in figure 4.2) via the ethynyl group. A monomer with one tag and a trimer with three tags symmetrically bound to a benzene ring were synthesized, the addition of more tags naturally enhances the absorption cross section. Experiments in the gas phase were only conducted with the monomer. Molecules were desorbed from a carbon wheel using IR-laser light at 1064 nm and pulsed injected into argon buffer gas with an Even-Lavie valve. Particles were postionized by VUV 157 nm laser light and extracted with a TOF-MS attached to a MCP. The cleavage mechanism was tested by co-aligning the 266 nm 4th harmonic of a Nd:YAG laser with the molecular beam to deplete the signal before postionization. The data was fitted with a modified formula for single photon absorption:

$$\frac{I}{I_0} = (1 - \alpha) + \alpha \cdot e^{-\sigma_{Pd} \cdot \Phi} \quad (4.12)$$

where I_0 is the signal from the parent molecule, I is the depleted signal, α is the beam overlap between the depletion and postionization laser, σ_{Pd} is the absorption cross section and Φ is the photon fluence. The data confirmed the successful implementation of a cleavage mechanism in the gas phase.

As a next step, PCTs again based on the nitrobenzyl group were attached to chains of 3 to 12 aminoacids, with the sequence Lys-Ala-(Leu-Gly-Ala) $_n$ -Leu², where $n = 0$ to 3. The peptide was designed to carry a singly negative charge and the PCT contains one of four different LGs[−], that should detach when a UV photon is absorbed. Two cleavage pathways were observed and are illustrated in figure 4.3: heterolytic cleavage enables neutralization by the separation of a negative LG[−] anion from a neutral peptide zwitterion. The second case involves cleavage of the LG under H-transfer which does not change the charge state of the peptide. The peptides were launched by ESI, mass selected by a quadrupol mass filter before they were guided through two hexapols. The first hexapole was filled with a buffer-gas (argon) to set the particle temperature in a range of 60-300 K. The cleavage mechanism was tested in the second hexapole by irradiating the particle cloud with a co-linear 266 nm laser pulse of 10 ns duration, at around 1 mJ. The particles were detected with a TOF-MS and MCP.

To learn more about the nature of the cleavage mechanism, the data collected by UV photodissociation (UVPD) were compared with data acquired by collision-induced dissociation (CID) during interaction of the peptides with the buffer-gas molecules at 300 K or 60 K. CID refers to cleavage of the peptide by collision and without light interaction. The authors found that for the case of the tripeptide ($n=0$) with LG-anion (a) in figure 4.3, heterolytic cleavage was the dominating pathway and that the UVPD and CID mass spectra were almost identical, indicating that the cleavage mechanism is of thermal nature. It was observed that one photon processes are dominant for molecules at 300 K, while for colder molecules at 60 K two photon processes are required, implying that the cleavage mechanism depends on molecular heat capacity. The heat capacity in return depends on the peptide length and it was observed that for longer peptides the cleavage mechanism shifts from heterolytic to predominately H-transfer. For CID, H-transfer was not observed however. Different LGs were tested to study heterolytic cleavage efficiency, i.e. charge stabilization of the electron on the LG. This mechanism was attributed to the fluorine atoms on the leaving group that have electron withdrawal and stabilizing properties, in agreement with the fact that for LG (c) heterolytic cleavage was less efficient. A drawback in the above described experiment is the fact that neutralization via heterolytic cleavage was only efficiently observed for small peptides.

An additional cleavage pathway was investigated in [102], with new LGs that enabled neutralization of more massive particles up to insulin. The general idea was to use either negative or positive charged LGs, that cleave in a homolytic fashion. When a single or multiple charged LG is cleaved of the molecule, the charge state is altered by the respective amount. This enables precise control over the charge state. The experimental setup is (almost) identical to the one described above. The LGs used were again based on fluorinated phenyls with additional either positive tetraalkylammonium LG⁺ or negative sulfonate LG[−]. Potential cleavage pathways and the different LGs are depicted in figure 4.4. The PCTs were again put on the same sequence of amino acids as described above, for the cases of $n=0$ (tripeptide) and $n=2$ (nonapeptide). The authors only observed homolytic cleavage and cleavage by H-transfer, but no heterolytic like in the previous experiment. Insulin was tested with three tags with LG[−]. For different initial charge states ($q = -2, \dots, -7$), cleavage was observed ranging from one up to all three tags, as well as electron photodetachment and a combination of both processes. For small initial charge states

²Abbreviations: Lysine, Alanine, Leucine, Glycine

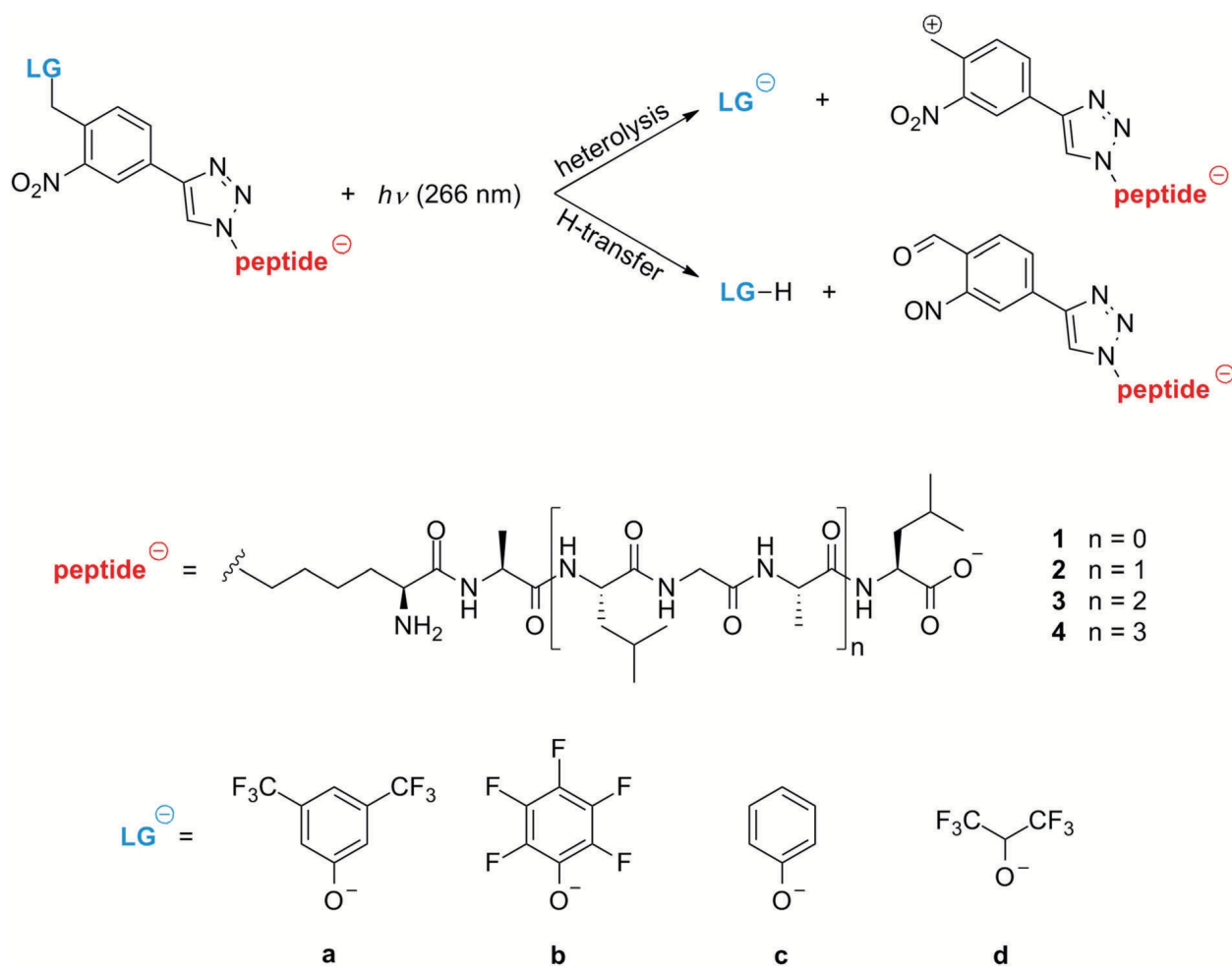


Figure 4.3: Possible cleavage pathways, peptide structure and LGs used in experiment [101]. Heterolytic cleavage splits of a negative LG under charge separation and neutralizes the initial negative peptide. Figure adapted from [101].

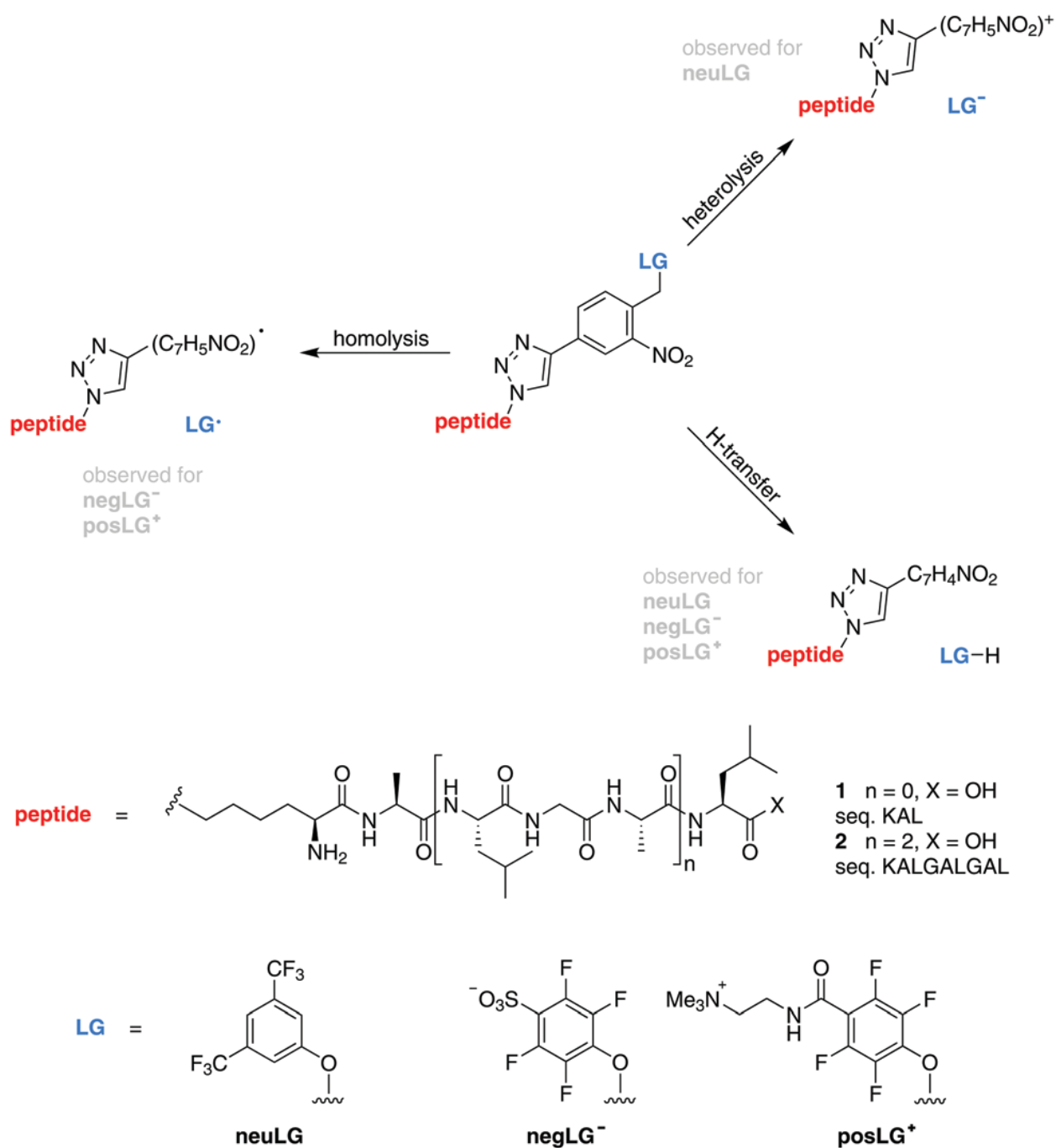


Figure 4.4: The tag can cleave from the peptide via three pathways depending on the LG. Homolytic cleavage results in the separation of the LG from the peptide under no charge transfer. The charge state of the total molecule is then reduced by the respective amount if a single positive or negative charged LG is used. Figure adapted from [102].

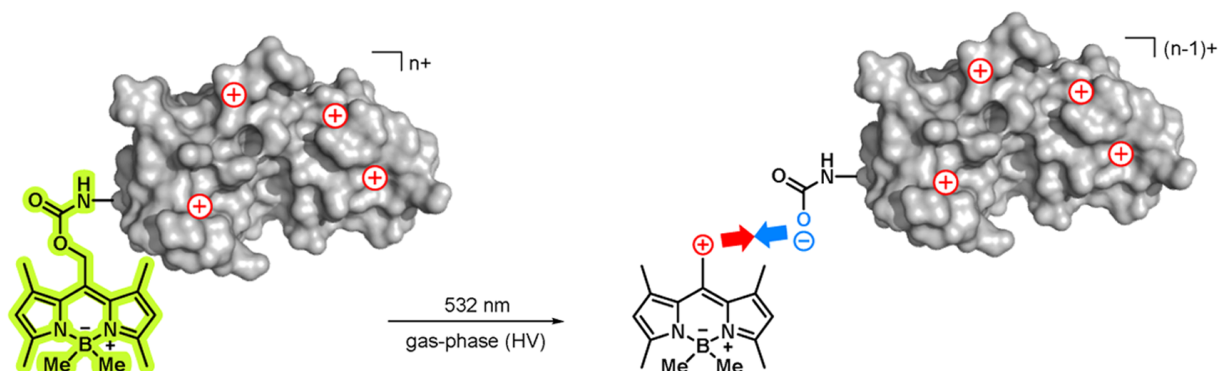
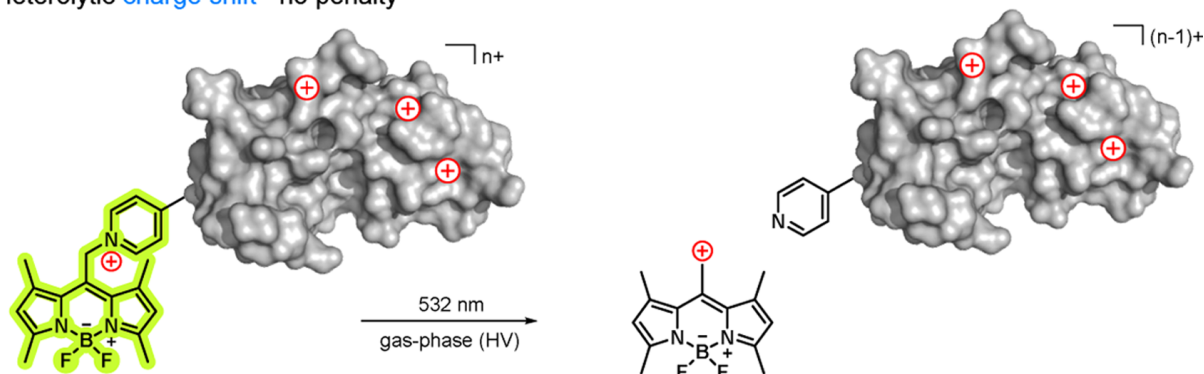
(a) Heterolytic **charge separation** - Coulomb penalty(b) Heterolytic **charge shift** - no penalty

Figure 4.5: Illustration of charge separation vs charge shift after green photon absorption. (a) 1-GGF cleaves heterolytically under charge separation but suffers from additional Coulomb attraction. (b) 2-OH shifts the charge from the pyridinium motif to the bodipy with no Coulomb attraction and reduces the charge of the parent molecule. Image adapted from [104].

$q < 4$, photocleavage was the only relevant process which demonstrates successful implementation of the technique. For us the most important result in this work is the neutralization of singly charged insulin by removing one of the three negative LGs. The authors successfully detected the negative LG with no signal of insulin in the spectrum for the case of UV radiation on the particle, which indirectly confirms neutralization. One could therefore combine this tag with a MALDI source that produces predominantly singly charged ions and neutralize particles before they enter the interferometer by 266 nm radiation.

4.2.2 Bodipy derivatives

Additional studies have been conducted that utilize green laser light with a wavelength of 532 nm to reduce charge states in the gas phase in [104]. The authors point out that absorption of amino acids like tryptophan, tyrosine and phenylalanine is reduced at this wavelength, leading to less absorption competition in comparison to 266 nm. Fragmentation is also reduced due to half the photon energy, however at the possible expense of efficiency because energy barriers are now more difficult to overcome. The Bodipy chromophore (figure 4.5) has well documented absorption at 532 nm and documented high uncaging ion yields in solution [158, 159]. Heterolytic cleavage was observed in polar solvents [160], which would allow charge reduction as indicated in figure 4.5 (a). Two bodipy derivatives were tested: bodipy with two additional methyl substituents attached to the bor atom with a *carbamate* linker termed 1-GGF (figure 4.5, (a)) and bodipy with fluor substituents and a pyridinium moiety termed 2-OH (figure 4.5, (b)). Both molecules

revealed interesting discrepancies between cleavage in solution and gas phase. In the gas phase, 1-GGF is excited to a singlet state after photon absorption with a lifetime of about 5 ns, but limited probability for photo processes due to a small Stoke shift (about 150 meV) and little excess energy [104]. Decay to a longer lived triplet states via intersystem crossing is improbable because of the absence of heavy atoms [159, 160]. The authors estimated that homolysis is the most probable process for cleavage of the carbamate from the bodipy, however the singlet state lifetime is too short to cross the energy barrier [161]. It was therefore expected that the state would decay into a singlet ground state via fluorescence or internal conversion, which could lead to heterolytic fragmentation when the photon energy is redistributed into the vibrational modes. However no cleavage was observed in the gas phase, contrary to solution. Fragmentation was however observed for CID with 5 eV nitrogen particles. This observation suggests that the photon energy of 2.33 eV was insufficient to surmount the dissociation barrier of the singlet state. This can be attributed to the Coulomb attraction between the charges, which exhibits greater strength in the gas phase as compared to polar solutions. Figure 4.5 (a) indicates this process.

This effect can be circumvented by a charge shift mechanism with lower activation energies for heterolysis [104]. Figure 4.5 (b) illustrates this effect for 2-OH, the charge moves from the pyridinium moiety to the bodipy fragment. Photolysis was not observed in solution, however charged bodipy fragments could be observed in high vacuum, with no signal for the neutral pyridyl-propionic acid residue, confirming the success of the mechanism. The tag was linked to insulin ($m \approx 5.8$ kDa), lysozyme and RNase ($m \approx 14$ kDa) via suitable linkers [162, 163]. A modified version termed 3-OH was attached to oxytocin, where fluorine groups replace the methyl groups on the bor atom in the bodipy group. The biomolecules were launched with an ESI source and mass selected in a QMS. They subsequently interact with the 532 nm cleavage laser at the end of a hexapole guide, with a variable laser fluence of 60-300 mJ/cm² at 10 ns pulse duration. Mass spectra are collected with a reflectron TOF-MS and MCP. The data confirms charge reductions by $-1e$: neutralization of oxytocin ($+1 \rightarrow 0$), as well as charge reduction for insulin ($+5 \rightarrow +4$), lysozyme ($+11, +10, +9 \rightarrow +10, +9, +8$) and RNase A ($+10, +9, +8 \rightarrow +9, +8, +7$). The experiment demonstrates an effective charge reduction mechanism, that could be utilized as a neutralization mechanism when biomolecules are launched with a MALDI or LDI source the could produce a high degree of singly charged molecules, which could subsequently be neutralized similar to the case of oxytocin. The bodipy tag would pose an additional advantage if the tag for diffraction at 266 nm does not couple to 532 nm, because the wavelength for neutralization is clearly distinguishable from the diffraction wavelength.

4.2.3 Porphyrin based test particles for photocleavage

We investigated three potential molecules as tags for matter-wave interferometry based on porphyrins (figure 4.6): Tetraphenylporphyrin (TPP), Diphenylporphyrin-4-(Dimethylamino) pyridin (DPP-DMAP) and Diphenylporphyrin-Tryptophan (DPP-TRP). TPP was purchased, DPP-DMAP and DPP-TRP have been specially designed for us by our Basel friends. Porphyrins can form large conjugated π -systems of more than 18 electrons that are considered aromatic [164] and exhibit strong absorption in the visible spectrum [165]. TPP consists of a porphyrin core structure with four additional phenyl groups attached and has a significantly high molar extinction rate of the order $\epsilon \approx 410000/(\text{cm} \cdot \text{M})$ at 419-422 nm wavelength [166]. DPP-TRP is a synthesized porphyrine with two additional phenyl groups and a modified tryptophan tag attached. DPP-DMAP has the same diphenylporphyrin core structure and a positively charged pyridinium tag linked to it. Both DPP-TRP and DPP-DMAP have a high resonance at 411 nm (figure 4.8).

This high absorption in the visible spectrum makes the porphyrin derivatives interesting can-

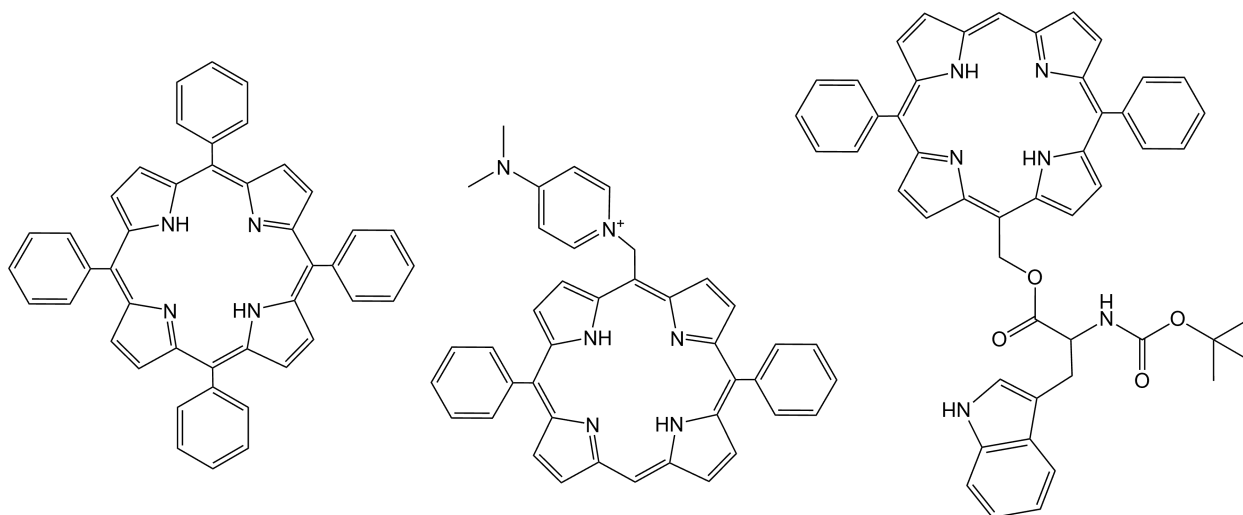


Figure 4.6: Three potential porphyrin based tag molecules, from left to right: TPP, DPP-DMAP and DPP-TRP.

didates for high efficient photocleavage, especially for potential neutralization and postionization mechanisms. For optical gratings the visible range from (411-420) nm is not preferable since first the scaling of T_L would increase at least by a factor of $(411/266)^2 \approx 2.4$ for equal λ_{db} wavelengths and for higher masses that is crucial. More important is the fact that 266 nm is a natural harmonic of commercially available powerful Nd:YAG lasers in contrast to 411 nm. The photon energy is additionally higher by about 1.6 eV for 266 nm. The porphyrines could therefore serve as tools for neutralization or postionization when attached to proteins, with a wavelength that is better distinguishable from the grating wavelengths. DPP-TRP and DPP-DMAP were designed to cleave under heterolysis to create a charged porphyrine fragment. However, the recent results from the bodipy experiment suggest that DPP-TRP can be expected to cleave homolytically from the carbamate in the gas phase, because the same Coulomb attraction between the negative oxygen and positive carbon hinders heterolytic cleavage. For DPP-DMAP, the charge transfer reaction similar to the 2-OH tag mentioned above is expected. The cleavage mechanisms and respective masses are illustrated in figure 4.7.

Possible applications in the case of heterolytic cleavage are for example: start negatively charged from MALDI-process to slow the molecules and then irradiate them with 411 nm to cleave of the TRP-tag and create a positive charged fragment that neutralizes the total protein. Another possibility is to use the same mechanism for postionization and detection after the tagged proteins pass the third grating neutrally. DPP-DMAP could be attached to a protein via the (dimethylamino)pyridin group that is initially charged to be desorbed (either by LDI or MALDI), slowed and then neutralized by heterolytic charge transfer of the charged DPP fragment, or as a postionization mechanism where the charged DPP stays linked to the protein. We know from previous OTIMA experiments that TPP fragments upon 422 nm radiation and cleaves of a phenyl ring. The high molecular extinction ϵ and commercial availability make it an intriguing PCT. However if cleavage occurs under homolysis the only option is usage in a recoil grating mechanism, which is not desirable for the reasons listed above.

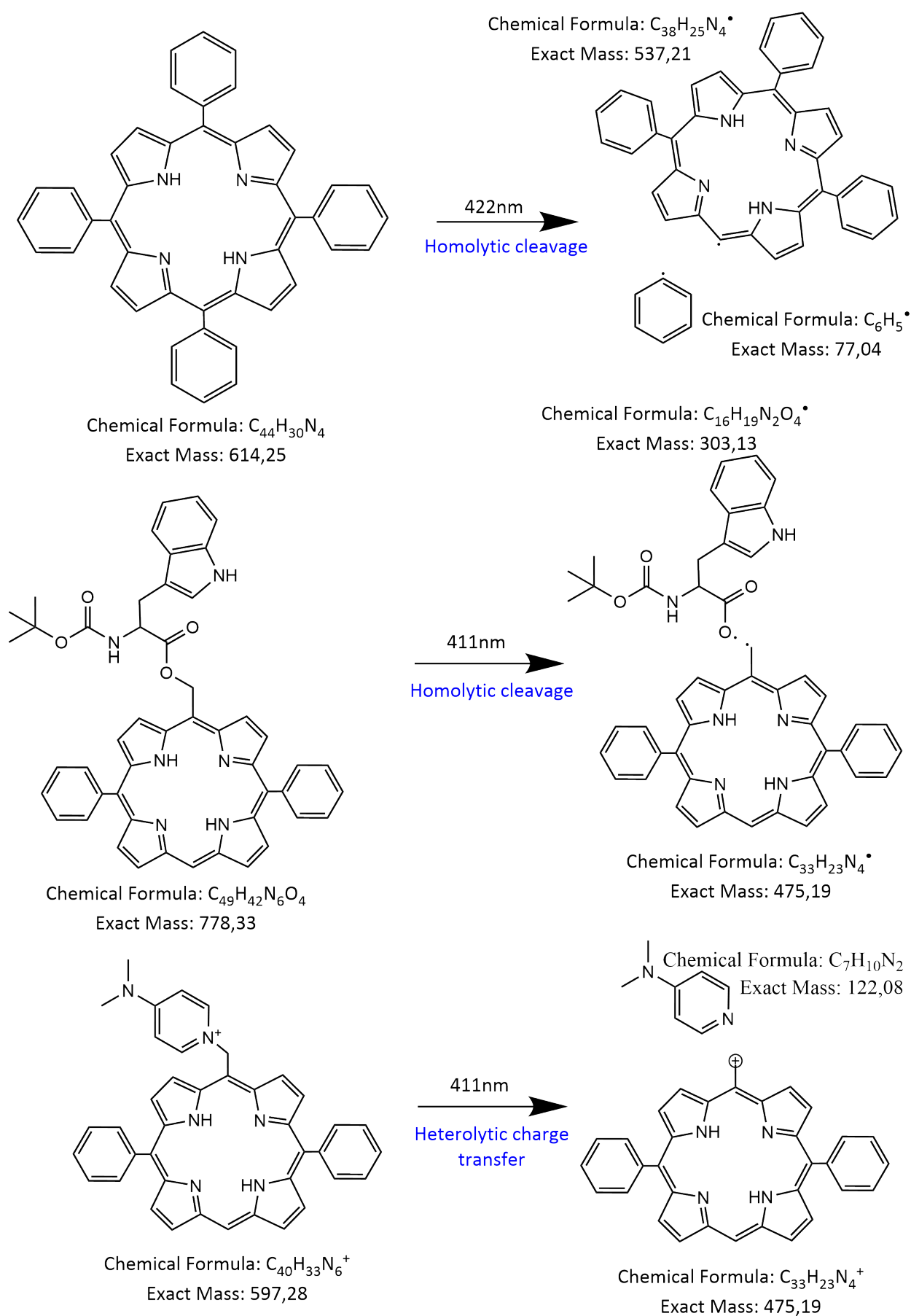


Figure 4.7: Expected cleavage mechanism that are experimentally investigated in section 5.6. The figure depicts the structure of the parent molecule on the left and the (heavier) fragments and (lighter) residues on the right after photon absorption.

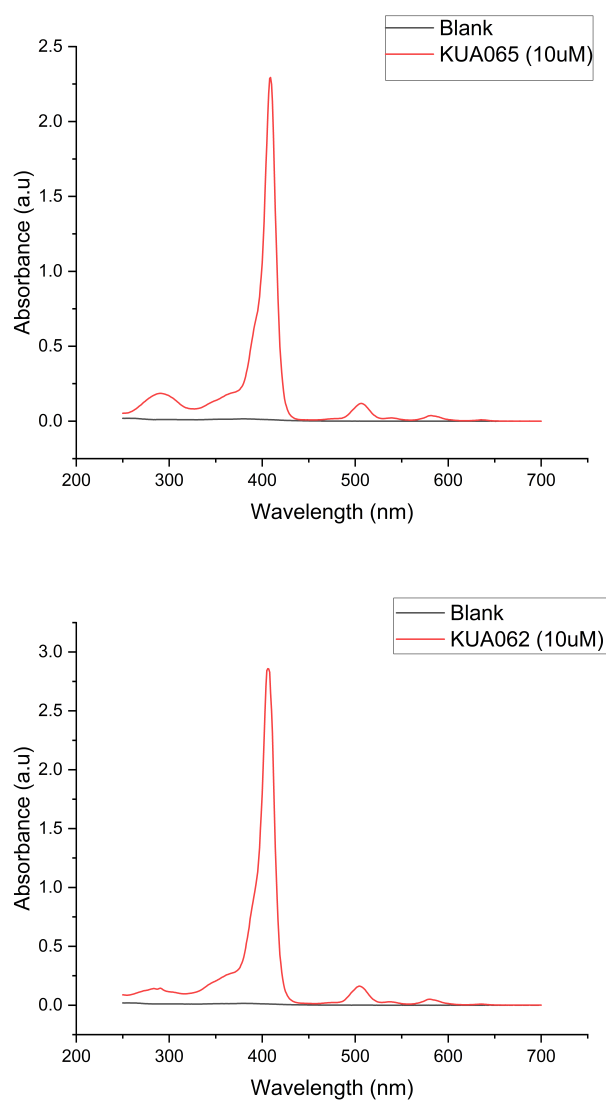


Figure 4.8: Absorption spectra for DPP-DMAP (upper) and DPP-TRP (lower) in solution. Spectra were provided by Basel.

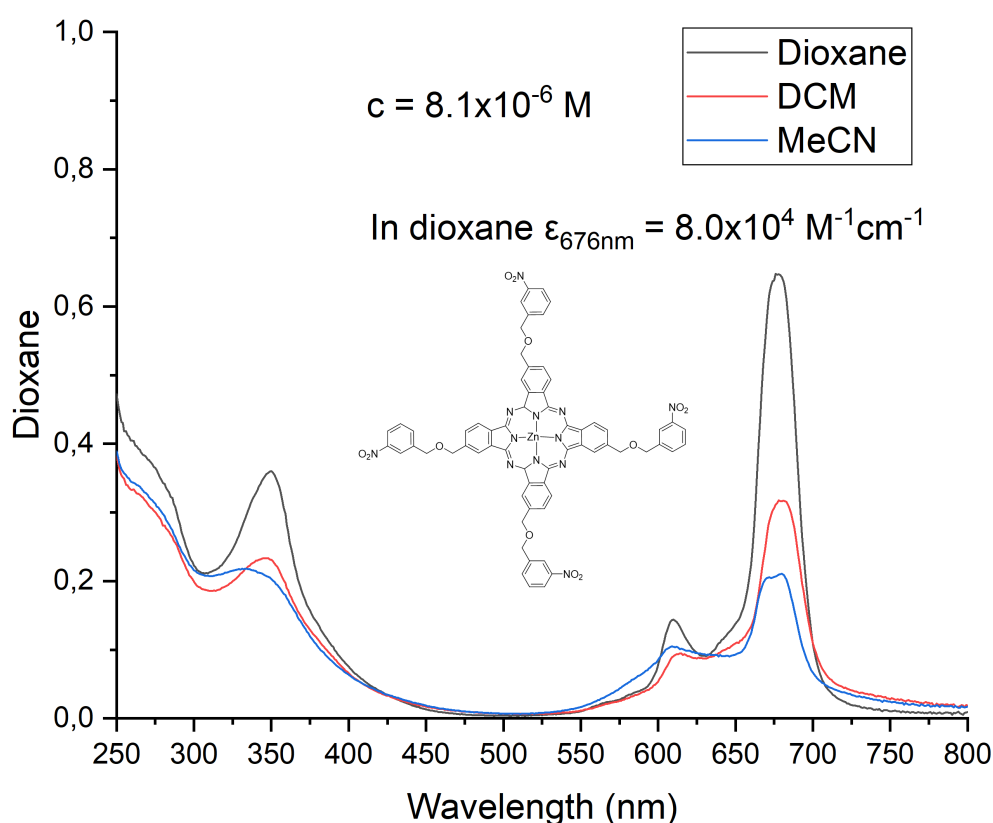


Figure 4.9: Absorption spectrum for zinc phthalocyanine functionalized with four nitrobenzylethers in different solvents. The spectrum shows high absorption in the visible spectrum for 676 nm and again increasing absorption in the UV-range. Spectrum was provided by Basel.

4.2.4 Functionalized Phthalocyanine

We also investigated zinc phthalocyanine functionalized with four nitrobenzyl-ether groups for photocleavage (PcH2-PCT). The structure ($\text{C}_{60}\text{H}_{36}\text{O}_{12}\text{Zn}$, exact mass: $m = 1180.1867 \text{ Da}$) and solution absorption spectrum are shown in figure 4.9. We saw in section 4.2.1 that the nitrobenzyl group can cleave under heterolysis and postionize the particle. The molecule exhibits high molecular extinction in the red visible range (in dioxane) $\epsilon_{676\text{nm}} = 8 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$. One could therefore use it as a potential tag for postionization after the interferometer, since the particle could be well targeted in this wavelength range without disturbing PCTs used for neutralization and diffraction. The increasing absorption in the UV-range also makes it an interesting candidate for diffraction at 266 nm by cleavage of the nitrobenzyl groups similar to the experiments discussed in section 4.2.1.

The central experimental task for the introduced test particles is to try to assign the expected masses after cleavage to peaks measured in the mass spectrum and thereby deduce the cleavage mechanism. The next task is then to characterize the efficiency of the process by measuring the dependence on pulse energy and extract the photon absorption cross section to determine if one or two photon absorption is required. The method is to fit the data to the exponential functions, for single photon with Eq. 4.2, for two photon with Eq. 4.5 or Eq. 4.11. Measurements and results are discussed in section 5.6.

Chapter 5

Experiment

The experimental setup is depicted in figure 5.1. A measurement process consists of the following steps: the sample is prepared on a target wheel, molecules are desorbed in vacuum by a laser pulse and orthogonal extracted by a TOF-MS. The ions are detected by a MCP and the signal strength in V is displayed against time on an oscilloscope. Pulse generators control the timing of various processes (section 5.5). Optional laser pulses with a OPO system for spectroscopy and a F_2 postionization laser can be provided. We will go through each step and the essential components in more detail in the following.

5.1 Sample preparation

Wheels of aluminum were used as a target, with diameters of $d = 4.5$ cm and a thickness of about $h = 3$ mm. For MALDI measurements, matrix and analyte solutions were prepared and mixed according to the direct mixing method described in section 3.1. The wheel is put on a small electrical engine to enable constant rotation. The direct mixed matrix/analyte solution is pipetted onto the outer surface of the wheel, until the whole surface is coated with a thin film of the sample solution. The effective surface is given by the laser spot size which depends on the focus (see figure 5.1, the general order of magnitude is $(100 \times 100) \mu\text{m}^2$). The surface can be scanned in the vertical direction by adjusting the height of the mirror governing the laser beam. Specific concentrations are given for the individual particles in section 5.6. The wheel is put into a small cube like chamber that is immediately closed and evacuated, to ensure rapid crystallization. Inside the chamber, the wheel is again mounted on a fixed electrical motor with the axis of rotation in the vertical z-direction. This way, the coated surface lies in the horizontal xy-plane of the chamber and can be ablated by the laser (figure 5.1).

5.2 Vacuum system and chamber

The whole chamber is under vacuum to avoid electric discharge between MCP channels and to ensure effortless passage of the test particles. A pre-vacuum rotary vane pump is connected to the chamber via a hose and a KF-flange, reaching pressure values of the order 10^{-2} mbar. Two turbomolecular pumps¹ connected via CF-flanges can be turned on to create a high vacuum. The four lateral surfaces of the cube like chamber can be opened and sealed via four CF-flanges that contain rubber sealing rings. The chamber can quickly be evacuated to perform sample exchange. Circular windows (glass, sapphire, CaF_2) can be added on the CF-flanges, to allow entrance of the lasers.

¹Pfeiffer Vacuum TC 600 and TC 110, 0-1500Hz

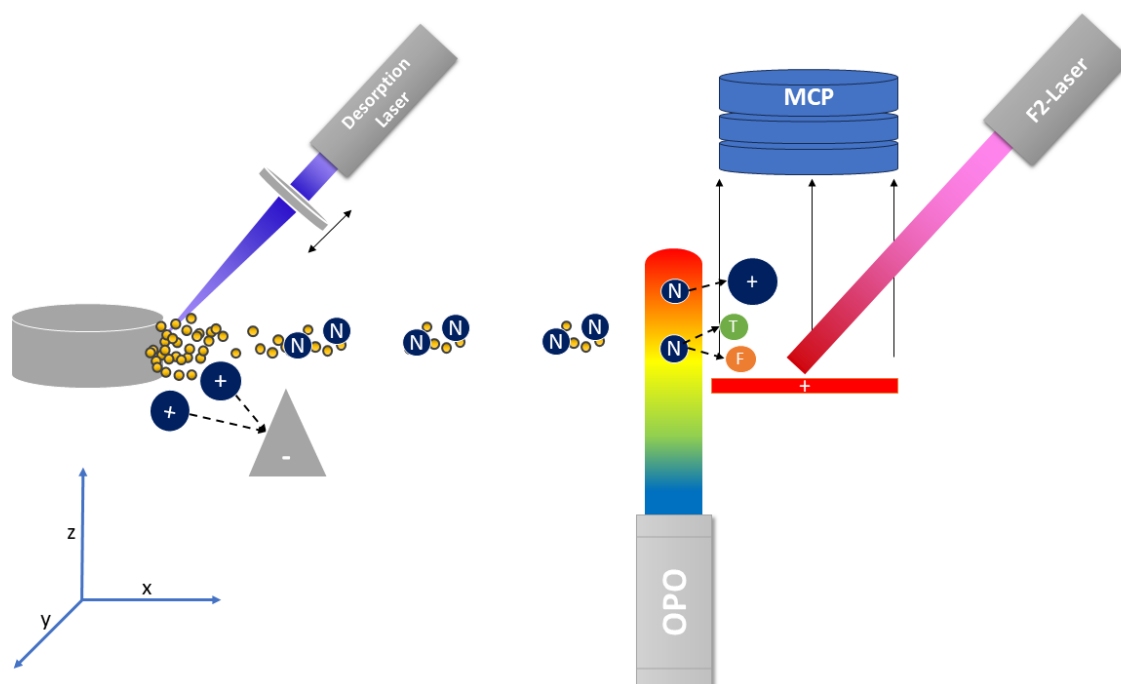


Figure 5.1: Sketch of the experiment. The sample is coated on a continuous rotating wheel, in crystallized form. The desorption laser (fs or ns pulse duration) is focused on the wheel ($f = 20$ cm), with the lens on a translation stage movable by ± 2.5 cm to change the spot size on the wheel. An electrode can optionally deflect or attract positive ions (+), with neutrals (N) passing through. Test particles can subsequently interact with a (unfocused) OPO pulse that irradiates the particles perpendicular to the propagation direction. Possible processes like ionization ($N \rightarrow +$) or photocleavage into fragment and tag ($N \rightarrow F + T$) are illustrated. An additional F_2 laser can be turned on to postionize particles in the repeller zone. Positive ions are deflected by the positive potential of the repeller electrode and directed to the TOF-MS and MCP for mass analysis. Only positive ions can be extracted so negative ions are not shown.

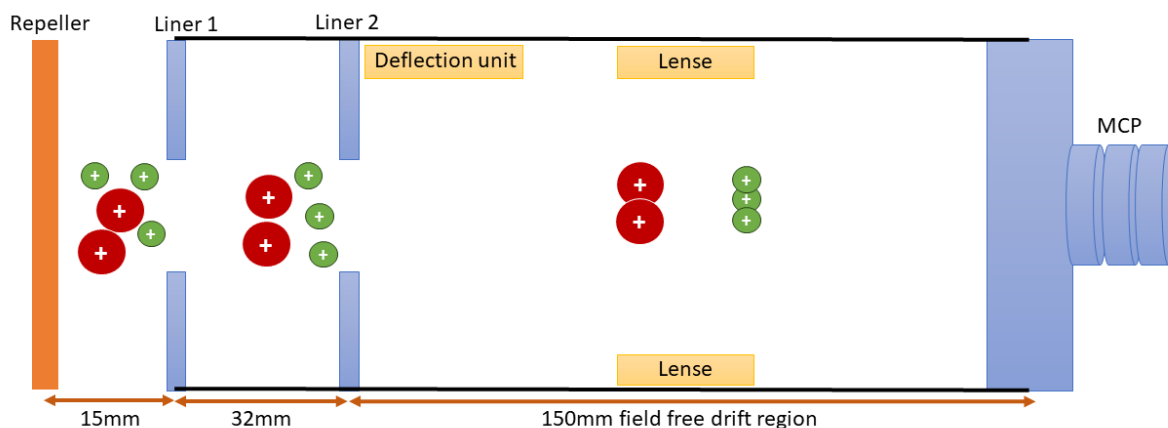


Figure 5.2: Sketch of the orthogonal TOF-MS used in the experiment. Heavier ions (red) are separated from lighter ions (green) by the 2 stage acceleration between the repeller, liner 1 and liner 2.

5.3 TOF-MS

A schematic sketch of the orthogonal TOF-MS² used for ion extraction is shown in figure 5.2. Particles are accelerated between repeller and liner 1. The system uses a two-stage extraction described in section 3.5, stage one is repeller-liner 1, stage two liner 1-liner 2. Only positive ions are deflected by the repeller potential. The repeller pulses are triggered with an external pulse generator, see section 5.5. After the ions are accelerated, they traverse a drift region where their trajectory can be influenced by additional orthogonal fields generated by a deflection element and an electrical lens. The deflection element compensates for the paths of heavier particles that would not make the turn because of their higher moment of inertia. The purpose of the lens is to focus the particle beam onto the MCP to create an amplified secondary electron signal that is recorded on an oscilloscope.

Calibration of the TOF-MS is based on the rearrangement of Eq. 3.1: $m/z = C \cdot t^2$, with a calibration factor that depends on TOF parameters and the flight time t for the ions from the repeller pulse to the MCP detector. The oscilloscope saves time data points with the trigger signal locked to the desorption laser pulse, see section 5.5. This yields $t = t_{raw} - t_0$, where t_{raw} is the data saved by the oscilloscope and t_0 is the starting time of the repeller pulse. The calibration factor was extracted by measuring the mass spectrum of a suitable substance with a well known mass distribution and linear regression to extract C . The substance of choice was caesium triiodide (CsI_3 , $m = 513.62$ Da), that clusters upon laser desorption to n^{th} order clusters of caesium iodine plus an additional caesium atom: $(\text{CsI})_n\text{Cs}$. The masses are $m_{\text{CsI}} = 259.83$ Da and $m_{\text{Cs}} = 132.91$ Da, thus mass values of $n \cdot (259.83 + 132.91)$ Da can be assigned. Typical TOF-MS settings are listed in table 5.1.

²Stefan Kaesdorf, Geräte für Forschung und Industrie

Electrodes	Repeller	Liner 1	Liner 2	Deflection Unit	Lense	MCP
Voltage [kV]	+1.98	-9k	-16.2	-6	-9	-2,6

Table 5.1: TOF voltage settings used for the majority of measurements.

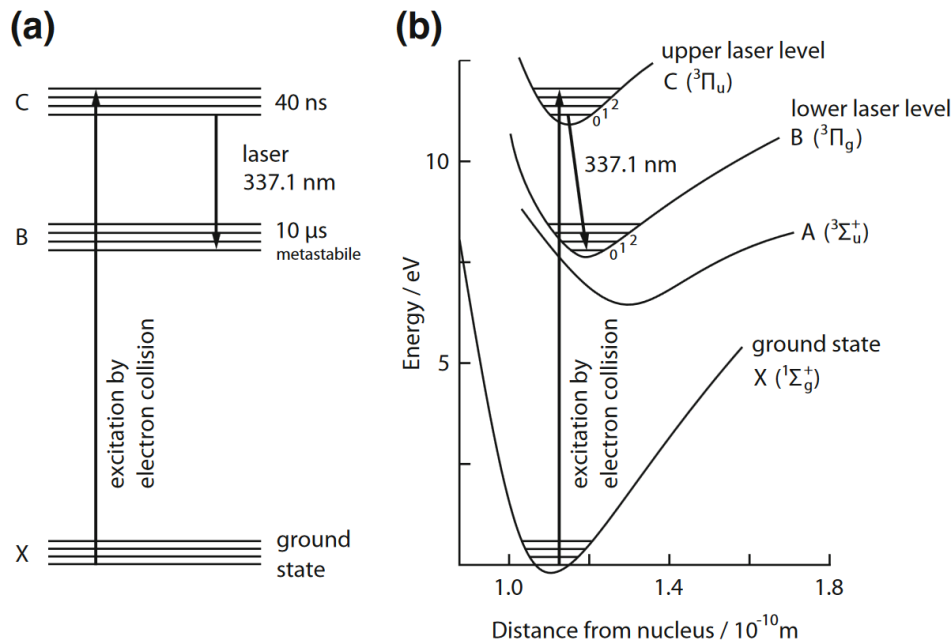


Figure 5.3: Nitrogen laser energy diagram. (a) Simplified version, the main transition occurs between the low vibrational electronic energy states C and B. (b) Detail version with potential curves of the involved states. Figure extracted from [167].

5.4 Laser systems

5.4.1 Nanosecond laser

One of the two optional desorption lasers was a nitrogen N_2 -laser³. The main transition line in nitrogen lasers emits at 337 nm, from the electronic transition $C (^3\Pi_u) \rightarrow B (^3\Pi_g)$ (figure 5.3). The first number labels the value of the main quantum number n , the upper left subscript is the total spin multiplicity $2S+1$. The projection of the total orbital angular momentum on the molecular axis is labeled Λ with values 0,1,2, $\hat{=}$ Σ , Π , Δ and the lower subscripts u, g refer to uneven or even parity. Vibrational modes of molecules are labeled with the quantum number ν , taking on values 0,1,2 and so forth. Both states involved in the main transition at 337 nm are in the vibrational ground state $\nu=0$ [167]. Population inversion from the ground state to the $C(^3\Pi_u)$ state is achieved by electron collision via pulsed gas discharges. This naturally leads to pulsed operation of the laser systems, with a pulse duration lying within a few ns and a maximum repetition rate of about 100 Hz [167].

In our experiments, the repetition rate was set to 50 Hz with a pulse duration of ≈ 4 ns. Pulse energies were measured at a maximum value of around 84 μ J, with the ability to attenuate down below the measurement sensitivity of < 14 μ J. The typical discharge geometry in nitrogen lasers creates a rectangular profile with high divergence and undefined polarization [167]. This in accordance with the experiments, the beam profile was approximately rectangular with a size of $A_L \approx (3 \times 7)$ mm measured at the position of the focusing lens.

³Laser Science, Inc

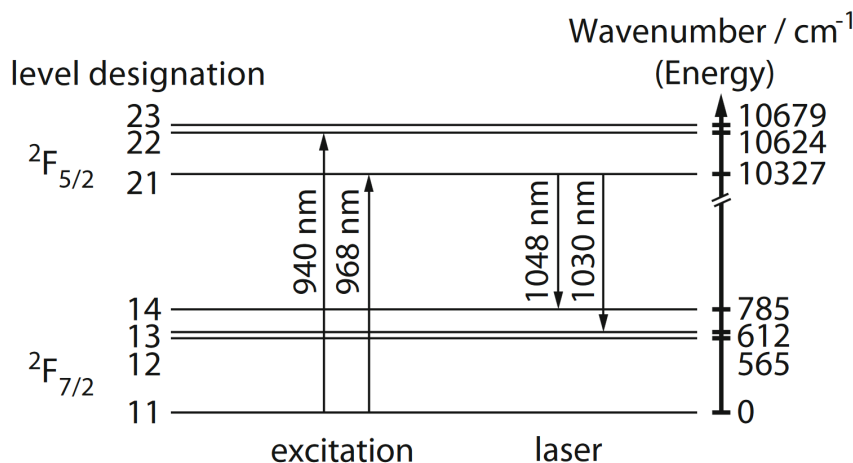


Figure 5.4: Energy levels and laser transitions for a Yb:YAG laser. Excitation at the absorption wavelengths 940 nm or 968 nm is achieved by diode pumping. The transition at 1030 nm is dominant for non selective resonators. Figure extracted from [168].

5.4.2 Femtosecond laser

A solid state laser system capable of fs duration laser pulses was alternatively used for desorption of molecules. Typically, the optical medium of a solid-state laser consists of a dielectric crystal doped with atomic ions that act as the gain medium [168]. In our experiments, two solid state laser systems were used based on the rare earth elements ytterbium (Yb) and neodymium (Nd), the latter is used in the OPO laser system, section 5.4.3.

The fs laser system used for desorption⁴ includes a Yb doped Potassium-Gadolinium-Tungsten (KGW, KGdW_2O_8) crystal as the gain medium, which is pumped by a laser diode. Atomic energy levels are labeled by the LS-coupling scheme: $^{2S+1}L_J$, where L is the total orbital angular momentum⁵, J the total angular momentum and $2S+1$ is again the total spin multiplicity. The laser diode drives transitions between the energy states $^2F_{7/2}$ (ground state) and $^2F_{5/2}$ (excited state) of the Yb^{3+} ions, where the electric field inside the crystal splits each energy state into sublevels labeled 11 to 23, according to the Stark shift [168]. The dominant line occurs between level 21 of the excited state and level 13 of the ground state at 1030 nm, which is the case for our laser system as well. Figure 5.4 depicts four sublevels of the ground state and three sublevels of the excited state for a Yb doped Yttrium aluminum garnet ($\text{Y}_3\text{Al}_5\text{O}_{12}$, YAG) crystal, however the energy level diagram and spectra are similar and representative for a Yb:KGW crystal [168]. A harmonic generator (harmonic generation, see appendix A) is fully integrated into the laser system and enables generation of laser light in the UV-regime at 343 nm (3^{rd} harmonic), suitable for laser desorption and MALDI. We operated the system in pulsed mode with a pulse duration below < 290 fs. An advantage of KGW crystals is the relatively high stimulated emission cross section in contrast to YAG crystals [168]. The output energy of the laser system can be adjusted with a tunable attenuator, the measured output energies were in range between 60 μJ , beyond the measurement threshold of < 14 μJ . The system was externally triggered (see section 5.5) to emit pulses of 100 Hz. A recent service report found that the beam profile is well described by a Gaussian beam and that the beam waists in x and y direction at the lens position are: $w_x = 3.23$ mm, $w_y = 3.33$ mm with M^2 values of $M_x^2 = 1.12$ and $M_y^2 = 1.09$.

⁴Pharos PH2-10W, light conversion

⁵L is labeled with S,P,D,F for $L=0,1,2,3$ and then continued alphabetically from G onward, J,S and P are often omitted in the notation.

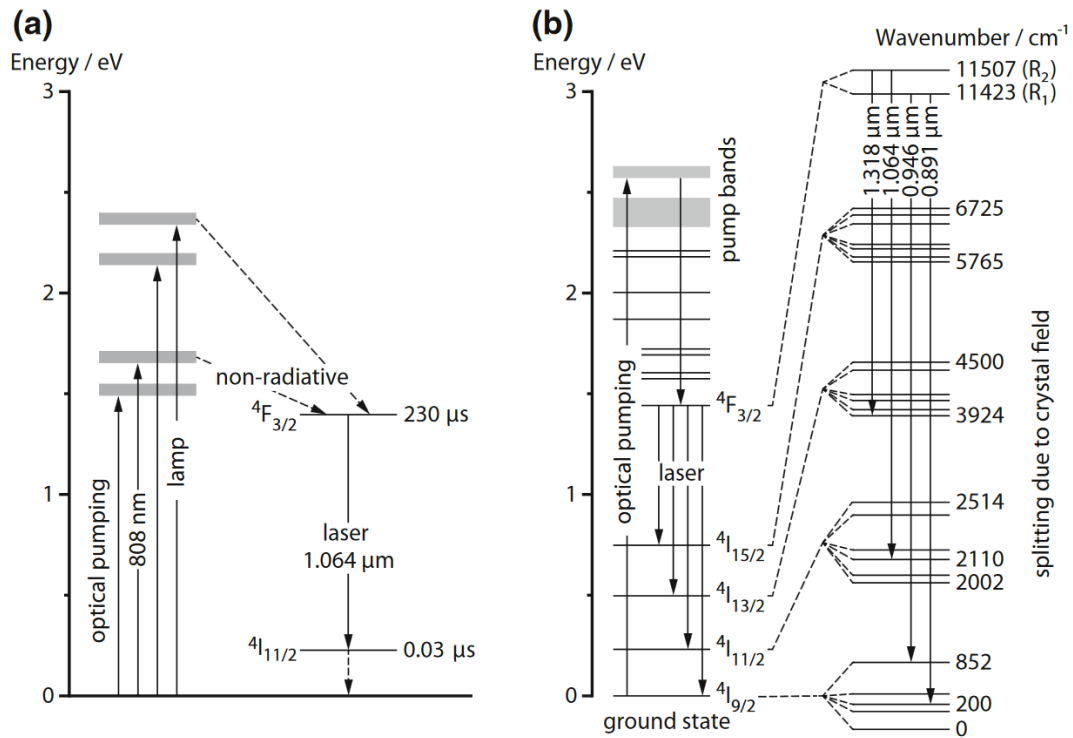


Figure 5.5: Energy levels and laser transitions for a Nd:YAG laser. (a) depicts a simplified version for the main transition. The system is first optically pumped, followed by a subsequent non-radiative decay to the excited state $4F_{3/2}$ that decays to $4I_{11/2}$ and enables operation at 1064 nm. (b) depicts a more detailed version with additional transitions. Figure extracted from [168].

5.4.3 OPO laser system

The laser system used for photon absorption of the PCTs is the NT242 OPO tunable pulsed Nd:YAG laser system of EKSPLA⁶. The medium here is a Nd doped YAG crystal pumped by a photodiode that drives the main transition between the $4F_{3/2}$ and $4I_{11/2}$ ($L = 6 \hat{=} I$) levels at 1064 nm, see figure 5.5. This wavelength is used to drive a complex system of non-linear optical elements that cover a wavelength range from (225-2600) nm. The physical principles behind non linear processes like second and third harmonic generation (SGH, THG) and optical parametric amplification and oscillation (OPA, OPO) can be found in the appendix A. The system has two outputs, the first one is for the third harmonic of the 1064 nm fundamental at 335 nm (this is also referred to as the pump beam since it additionally serves as the pump source for the OPO system). The energy of the pump is monitored by a photodiode and is displayed on the controls for the laser system. In our experiments, the pump energy was around (37-41) mJ/pulse, operated at a repetition rate of 100 Hz with pulse duration of the order ≈ 5 ns. The second output covers the total range of (225-2600) nm and consists of a system of non linear Beta-Barium Borate ($\text{Ba}(\text{BO}_2)_2$, BBO) crystals. The conversion leads to energy loss, resulting in a variation of the pulse energy over different wavelengths at this output. The output energy is additionally varied by a Fresnel-Rhomb (FR) and a polarizing beam splitter (PBS), or a neutral-density (ND) filter for 266 nm measurements. The FR changes the polarization state of a certain number of photons and the PBS transmits only parts of the photons in the laser pulse and thus less energy. The beam profile is Gaussian and approximately circular, the diameter was estimated on a burn patten to about $d \approx 3.73$ mm.

⁶Manufakturer of laser systems, Lithuania

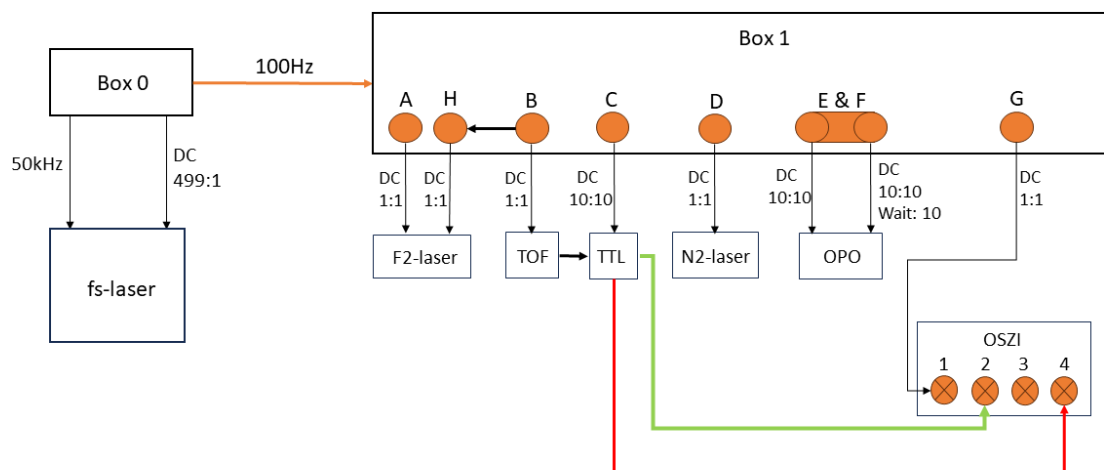


Figure 5.6: Sketch of triggering/timing. The individual components are described in the text.

5.4.4 Excimer laser

For postionization an excimer gas laser was used. The word excimer is an abbreviation for excited dimer, since usually the gain medium for this type of lasers consists of noble gas dimers in an excited state, like argon, krypton or xenon: Ar_2^* , Kr_2^* or Xe_2^* [167, 169, 170]. No stable ground state exists for these molecules. However, the excited state of the molecule is weakly bound with a lifetime on the ns scale and can lead to spontaneous or stimulated emission of a photon with a specific frequency, when the state decays back in the unstable ground state. Population inversion into the excited state is usually achieved by electric gas discharge pulses. Today, the term also incorporates not only dimers of noble gases, but also so called *exciplexes* of molecules consisting of a noble gas atom and a halogen atom, or two halogen atoms. A prominent example is the Krypton-Fluor laser (KrF)^{*}, emitting at 248 nm [169].

In our case, we used an excimer laser system⁷ filled with difluorine F_2^* as the gain medium, emitting in the UV regime at 157 nm. At this wavelength, oxygen in the atmosphere absorbs the laser radiation after fractions of a millimeter, so vacuum beam lines would be required. We used a short line filled with pure nitrogen gas. The laser enters the chamber directly without any further optics. It has a maximum pulse repetition rate of 500 Hz, a duration of about 5 ns with used pulse energies in the range of (1.5 - 1.8) mJ. The laser emits a rectangular flat top profile where the size was adjusted both vertically and horizontally with collimation slits before passage through a CaF_2 window into the chamber.

5.5 Triggering and timing of the experiment

Figure 5.6 sketches the timing and triggering of the experiment. The timing of the MALDI setup is controlled via a BNC-pulse generator (box 1). This box is triggered by another BNC-box (box 0) with a frequency of 100 Hz to form the basic time unit for a measurement cycle. Since for

⁷Coherent, Exci Star XS 500

technical reasons the OPO laser systems works best when operated at consistent 100 Hz, but the nitrogen can only operate with 50 Hz, we adjusted the duty cycle mode of several channels to 1:1, to split the 100 Hz signal to 50 Hz. Box 1 has channels (CH) A to H that can transmit pulses. Two properties of these pulses can be directly adjusted at the box, the pulse width (WDT) that specifies the duration of one pulse and the pulse delay (DL). The DL adjusts the timing between the initial pulse box 1 receives by box 0 (with 100 Hz) and the initiation of the pulse created by box 1 on the respective channel. Figure 5.6 sketches the timing with the channels and the units they trigger. Box 0 is necessary to drive the oscillator of the fs laser with 50 kHz. The pulse repetition rate was set to 100 Hz with a duty cycle (DC) of 1:499. Two signals are necessary to trigger the F₂ laser. CHA transmits a "ready" signal to the laser with 50 Hz by setting the DC to 1:1 and CHH supplies the "fire" signal that initiates the gas discharge in the laser. CHB triggers the repeller voltage of the TOF-MS with 50 Hz. Pulse DL and WDT of CHB specify when the repeller is turned on and for how long it stays on. The timing of CHH was set relative to CHB (B → H, fig 5.6) to enable postionization in synchronisation with the repeller pulse. CHD triggers the N₂-laser with 50 Hz and CHG triggers the oscilloscope (OSZI).

For OPO measurements, it is convenient to depict the pure desorption signal without interaction by the OPO laser pulses on input 2 on the OSZI and the signal with interaction on input 4. To better visualize the effect, we designed the experiment such that the OPO only interacts with every second particle pulse. The two cases with OPO interaction (OPO) and without (NOPO) are simultaneously depicted on the oscilloscope at input 2 and 4. We modified the OPO timing such that the laser pulse is resonant with the particle cloud only every second pulse. To achieve that, CHE and CHF of box 1 are set to multi channel mode, so that the OPO system is triggered by both channels. Both are put into a duty cycle mode of 10:10, but CHF is shifted by an additional setting to wait the first 10 pulses. The result is that the first 10 pulses are delivered with CHE settings and the next 10 pulses are with CHF settings. CHE is on time, i.e. adjusting the delay so that the OPO is resonant with the cloud and CHF is off time. To visualize the two signals on the oscilloscope, CHC of box 1 is also put into 10:10 duty cycle mode and connected to a TTL-switch⁸ with two output ports. The TTL-switch transmits a signal in either output port 1 or 2, whether it receives a signal from CHC or not, which is provided by the duty cycle mode. The TOF signal recorded by the MCP is directed towards the TTL-switch. Since CHC, CHE and CHF are set to equal duty cycle modes of 10:10, the TTL-switch works in synchrony with the OPO. When the OPO is on time, the TTL-switch receives a voltage signal and transmits the TOF signal in one output port. When the OPO is off time, there is no signal arriving from CHC and the TTL-switch transmits the TOF signal in the second output port. The two signals are directed to inputs 2,4 and simultaneously displayed. The two signals with and without OPO resonance can be easily compared that way.

⁸Transistor–transistor logic

5.6 Results and discussion for PCTs measurements

The following section discusses measurements conducted for TPP, DPP-TRP, DPP-DMAP and PcH2-PCT.

5.6.1 Tetraphenylporphyrin

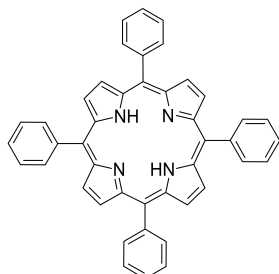


Figure 5.7: TPP

The sample was initially embedded in felt and subsequently rubbed on the target wheel without solution or matrix. We studied the molecular velocity for pure LDI both for ions created in the desorption process and for neutrals postionized by the F_2 laser. Velocity distributions were measured for ions and for neutrals by dividing the flight distance from the wheel to the source $\Delta x \approx 4$ cm by the travel time t . The travel time can be estimated by $t = (D_{CHB} - D_{CHG}) \cdot 10^{-6}$, where D_{CHB} is the channel delay in μs for the repeller pulse and D_{CHG} is the start trigger for data capture on the OSZI, which occurs concurrently with the laser pulse. The difference is equal to the time of flight from the wheel to the center of the extraction zone. Velocity measurements for

ions and neutrals are plotted in figure 5.8 with the respective desorption conditions. Ion production for LDI seems to only occur in the upper layers of the sample, a stable signal for a new spot could only be recorded for a few minutes after irradiation, whereas neutrals could be detected from the same spot for hours, with a ten times stronger signal. Ions propagate about three times faster than neutrals according to figure 5.8.

Photodepletion measurement. Absorption of photons in TPP can result in cleavage of a phenyl ring. The depletion of the main signal was measured as a function of the pulse energy to deduce if the ring cleaves from a one or multiple photon process. The depletion measurement was performed for neutral particles with subsequent postionization at 157 nm. Depletion and photon absorption curves are shown in figure 5.9. The depletion of the main peak was fitted with a combination of a REMPI (1+1) process (Eq. 4.11) and the beam overlap parameter (Eq. 4.12):

$$\frac{N(E_P)}{N_0} = 1 - A - \frac{Ae^{-(s_1+s_2)E_P/(2A_LE_\gamma)}(s_1 - s_2)}{2s_2} + \frac{Ae^{-(s_1-s_2)E_P/(2A_LE_\gamma)}(s_1 + s_2)}{2s_2} \quad (5.1)$$

with the relative depletion signal (OPO/NOPO) given by $N(E_P)/N_0$, the photon energy E_γ , the laser pulse area A_L and the fitting parameters A, a and b, where $s_1 = 2a+b$ and $s_2 = \sqrt{4a^2 + b^2}$. The REMPI (1+1) cross sections $\sigma(1)$ and $\sigma(2)$ can be extracted from the fit:

$$\sigma(1) = (2.82 \pm 0.13) \times 10^{-16} \text{ cm}^2 \quad (5.2)$$

$$\sigma(2) = (5.63 \pm 0.34) \times 10^{-16} \text{ cm}^2 \quad (5.3)$$

The uncertainties were determined based on the uncertainty from the fit (figure 5.9 (2)). The data fits a REMPI (1+1) and homolytic cleavage process. No charged fragment or phenyl ring residue could be detected when the postionization laser was turned off. Although efficient cleavage could be demonstrated, the apparent homolytic cleavage process renders the particle not suitable for neutralization as well as for the grating mechanism, since 422 nm is not provided by the Nd:YAG harmonics and would additionally imply a $221^2/133^2 = 2.8$ times larger Talbot-Length T_L for in contrast to 266 nm if used for the grating.

Postionization for 266nm

Pure LD was used for desorption of neutral TPP molecules with selected particle velocities at $v = 667$ m/s. No ions could be detected from the desorption process alone with a laser pulse energy



Figure 5.8: Measured velocity distributions for ions and neutrals of TPP. Neutrals were desorbed with 343 nm wavelength, 290 fs repetition rate and $E_P \approx 10 \mu\text{J}$ pulse energy focused to the wheel with a fluence of $F = 4.68 \text{ mJ/cm}^2$. The F_2 laser postionized the particles with 157 nm wavelength and 1.58 mJ pulse energy. Ions were desorbed with a different pulse energy of $E_P \approx 15 \mu\text{J}$ and focus, with a fluence of $F = 4.81 \text{ mJ/cm}^2$.

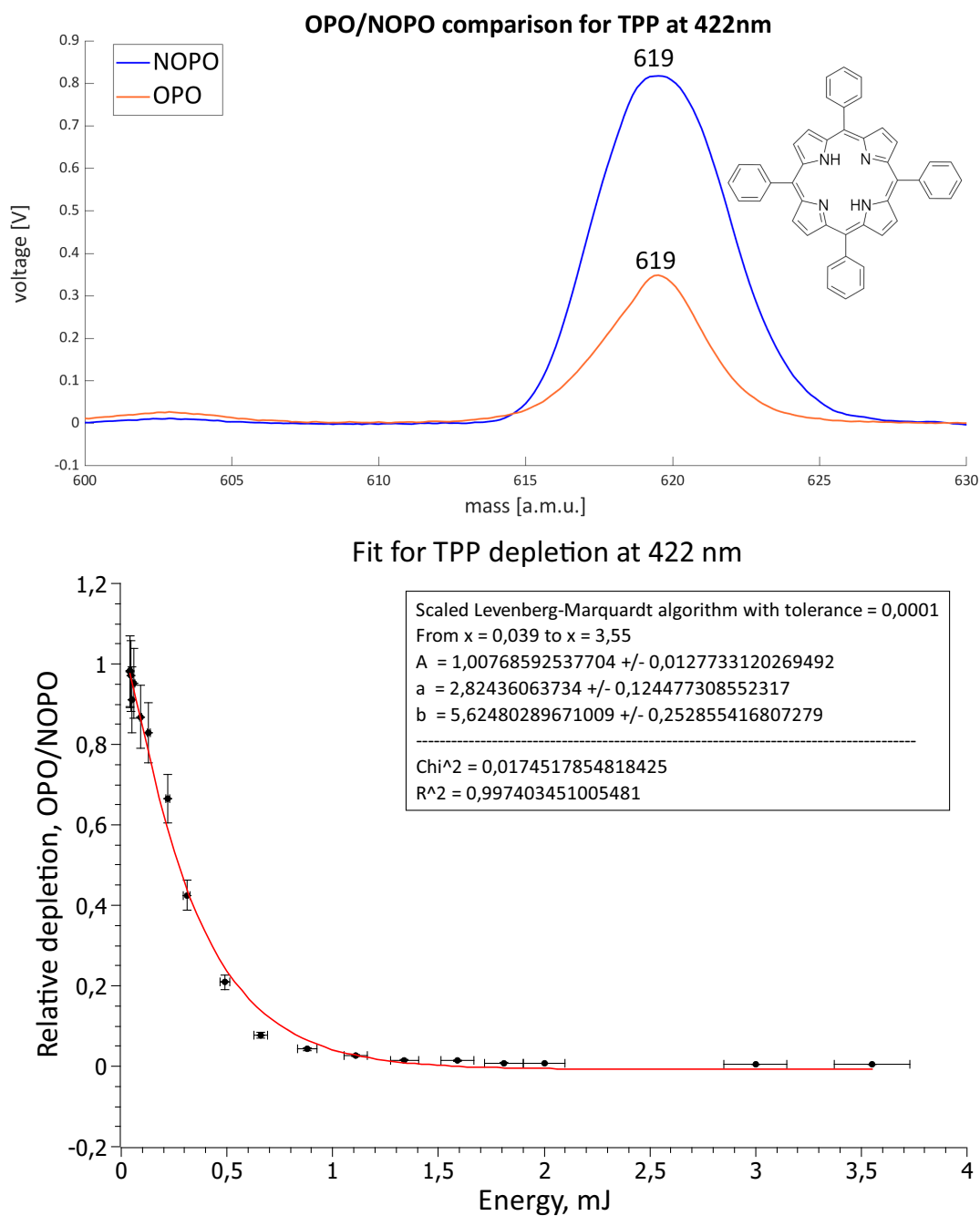


Figure 5.9: (1) Example mass spectrum to illustrate the cleavage effect. The x-axis is in mass units since $z = 1$ for all peaks. Irradiation with 422 nm reduces the signal (OPO, here $E_P = 0.3$ mJ). The shift from 614 Da to 619 Da in the mass spectrum can be attributed to a slightly inaccurate timing calibration between the TOF repeller pulse and the postionization laser at the time of the measurement. (2) Photodepletion curve for increasing pulse energy. Error bars for the x-axis (5 %) result from fluctuations of the laser power observed during measurement, for the y-axis ($1/\sqrt{128} \approx 9$ %) they result from shot averages.

of $E_{337} \approx 16 \mu\text{J}$ and corresponding fluence at the wheel $F = 21 \text{ mJ}/\text{cm}^2$. Irradiation of the cloud with 266 nm by the OPO resulted in signal detection of TPP at $m = 614 \text{ Da}$ (mass spectrum in figure 5.10) which is interpreted as postionization of TPP molecule changing the charge state to $z = +1e$. The TPP ion yield was plotted as a function of pulse energy in figure 5.10, (2)) to deduce the nature of the absorption process. Fitting the data with a resonant two-photon process was not successful, the data favors an off resonant process (figure 5.10) with the function from Eq. 4.5:

$$N_i(E_P)/N_0 = (1 - e^{-bE_P^2}) \quad (5.4)$$

where $N_i(E_P)/N_0$ is the relative ionization signal, E_P the pulse energy and $b = \sigma_2/((E_\gamma \times A_L)^2 \times \tau)$ is a fitting parameter that contains the cross section σ_2 , the laser pulse area A_L , the photon energy E_γ and the laser pulse duration τ . The off resonant 2 photon cross section is extracted from b and results in:

$$\sigma_2 = (4.33 \pm 0.13) \times 10^{-41} \text{ cm}^4\text{s} \quad (5.5)$$

The uncertainty results from the fit uncertainty and error propagation. The saturation threshold of absorption could not be reached with the available pulse energies. Higher pulse energies would be required to determine saturation, however it seems that the fact it is a 2 photon process does not make this particle a suitable candidate for the grating mechanism.

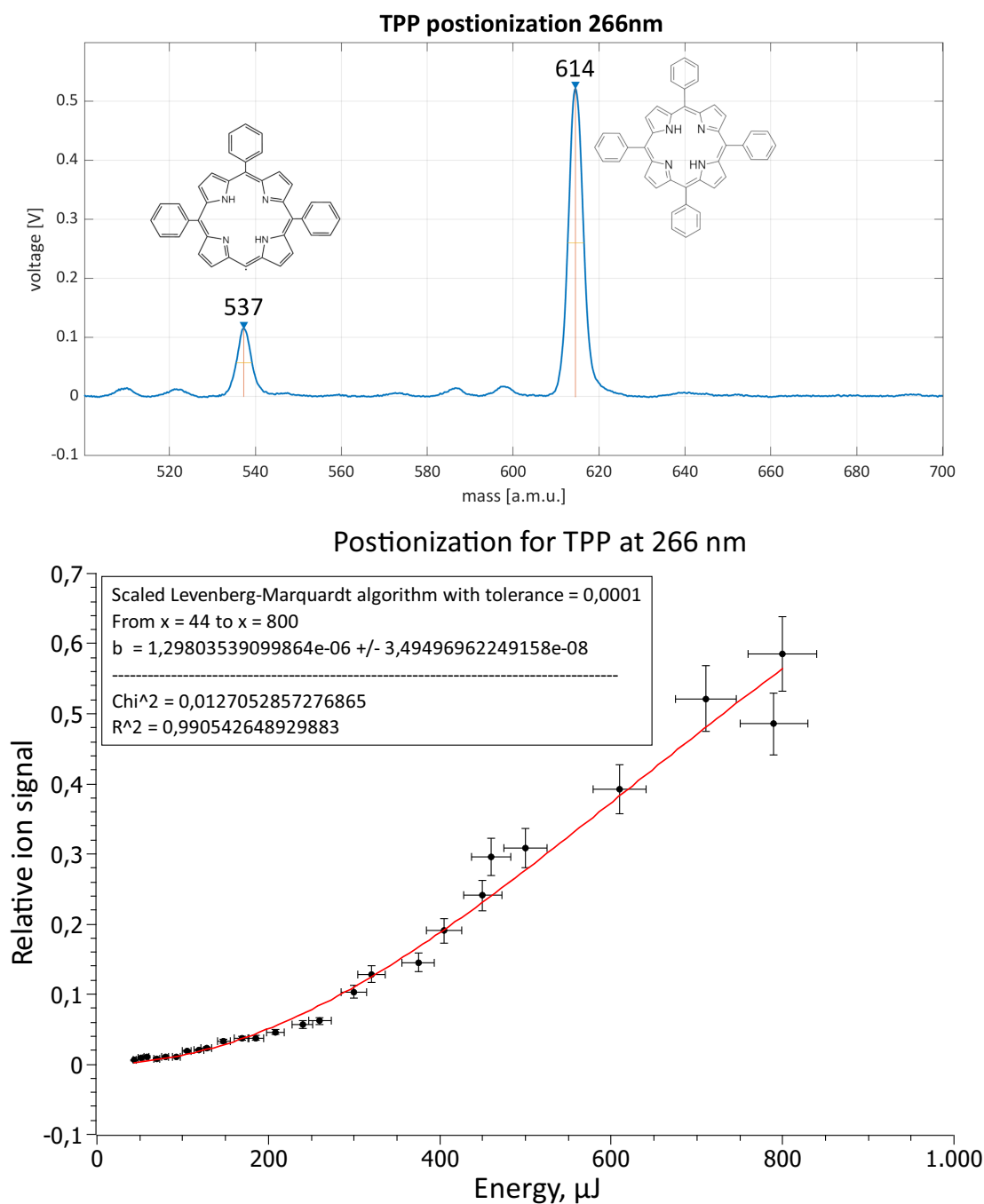


Figure 5.10: (1) example of a mass spectrum of postionized TPP ($m = 614$) and TPP minus a phenyl ring at $m = 573$. (2) Signal of the main peak plotted against pulse energy. The data was fitted with an off resonant 2 photon absorption curve. The signal error was quantified with $1/\sqrt{128} \approx 9\%$ and the error for the E_P with 5 % from the observed laser power fluctuation during measurement.

5.6.2 DPP-TRP

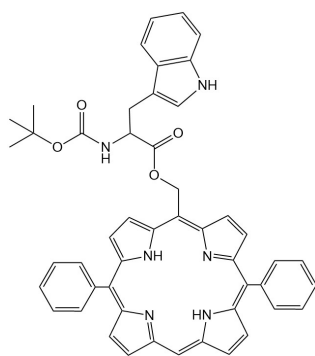


Figure 5.11: DPP-TRP

An amount of 5 mg of CHCA in 40 μl DMSO and 0.5 mg of DPP-TRP in 10 μl DMSO has been prepared. 5 μl of analyte + 40 μl matrix were mixed together to produce a final matrix/analyte ratio of $R \approx 100 : 1$ on the target. The solid sample was desorbed with a 290 fs pulse duration and 343 nm wavelength, with a pulse energy of $E_{343} \approx 12 \mu\text{J}$ focused to a fluence of $F = 9.16 \text{ mJ}/\text{cm}^2$. Ions were deflected with 140 V and neutrals were studied by postionization with 1.7 mJ pulse energy at 157 nm wavelength. The velocity was selected to $v \approx 400 \text{ m/s}$.

Three components could be identified in the mass spectrum (figure 5.12) that fit the main peak at $m = 776 \text{ Da}$, the DPP fragment plus carbon (DPP + CH_2) at $m = 462 + 14 = 476 \text{ Da}$ and the tag residue at $m = 303 \text{ Da}$. The assignment of the main peak is slightly ambiguous since it is off by 2 mass units, however, the TOF has an uncertainty of $778/500 = 1.6$ in this range and the particle could potentially be missing one or two hydrogen atoms. The measured mass spectra indicate cleavage already at the desorption stage and generation of neutral fragments that were detected after postionization with 157 nm. No signal could be detected without the postionization laser. Cleavage was tested by irradiation with 411 nm with the OPO and by variation of the pulse energy with the FR and PBS system. The data acquired seem to support homolytic cleavage. The DPP-TRP signal at $m = 776 \text{ Da}$ was depleted under radiation and thereby demonstrating cleavage, figure 5.12 (1) shows the depletion of the main peak. The signal for the DPP + CH_2 fragment at $m = 476 \text{ Da}$ is increasing for initial pulse energies but again reduced for higher pulse energies (figure 5.13), which we interpret as homolytic cleavage of one of the phenyl rings, similar to the TPP case. The tag signal at $m = 303 \text{ Da}$ seems to be independent of the OPO and remained constant over time. The depletion of the main peak was fitted with a (1+1) REMPI process (figure 5.12 (2)), similar to TPP. The (1+1) REMPI cross sections $\sigma(1)$ and $\sigma(2)$ can again be extracted from the fit:

$$\sigma(1) = (4.41 \pm 0.17) \times 10^{-16} \text{ cm}^2 \quad (5.6)$$

$$\sigma(2) = (8.87 \pm 0.34) \times 10^{-16} \text{ cm}^2 \quad (5.7)$$

The uncertainties were determined based on the uncertainty from the fit (figure 5.12 (2)).

We first considered a heterolytic cleavage process of the DPP-TRP particle into a positively charged DPP + CH_2 fragment and a negatively charged tag residue. A signal of the DPP + CH_2 fragment was recorded without the 157 nm postionization laser to look for the positive fragment in case of heterolytic cleavage. A signal could be observed, however, it does not seem to match the expected behaviour for heterolytic cleavage, since the signal rises with increasing pulse energy without the signal dropoff observed before in figure 5.13. This indicates a potential multi-photon ionization mechanism for the fragments that already exist in the beam. In this case, the OPO would cleave the DPP-TRP homolytically and cleave off additional phenyl rings, but those events are not observable since they do not result in a change of the charge state. Only DPP+ CH_2 fragments that already exist in the beam and are postionized by the OPO with a multi-photon process. The result is a steady increase of the signal until saturation. Saturation was not observed for the available power since a higher photon density is required in the case of a multi-photon process. Attempts were made to fit the data with an appropriate function, but so far they were not successful. So the interpretation of the data is not unambiguous, it can only be concluded that the depletion of the main DPP-TRP particle does involve a (1+1) REMPI process, but the nature of the cleavage mechanism remains an open question, although homolytic cleavage seems

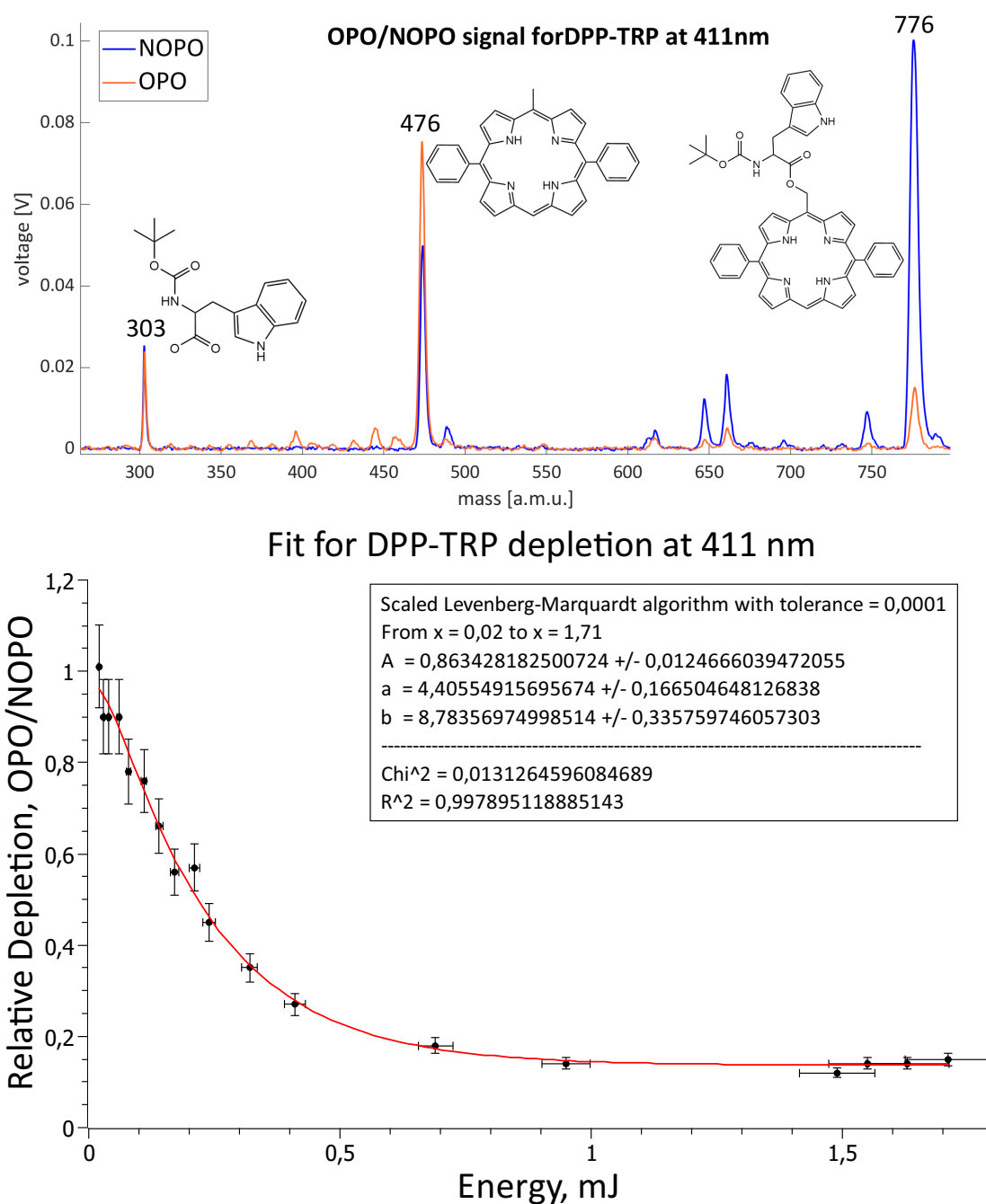


Figure 5.12: (1) Example mass spectrum for a photodepletion measurement at 411 nm and 1.7 mJ pulse energy (OPO). We identify the main signal at 776 Da, DPP+C at 474 Da and the tag at 303 Da. (2) Signal of the main peak plotted against pulse energy. The data was fitted with a (1+1) REMPI photon absorption curve. The errors were again quantified with 5 % for the x-axis and 9 % for the y-axis.

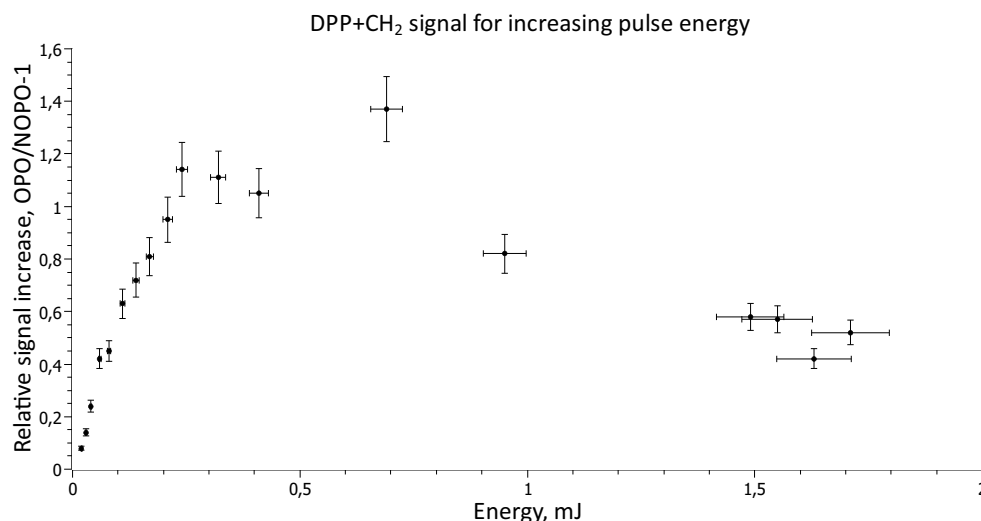


Figure 5.13: Signal dependence of DPP+CH₂ fragment (476 Da) for increasing pulse energy. The signal increases for lower pulse energies and gets depleted again for higher pulse energies. This is interpreted as cleavage of a phenyl ring. The errors were again quantified with 5% for the x-axis and 9% for the y-axis.

more plausible. A TOF-MS with the ability to switch to a negative extraction mode could be used to search for the negatively charged tag signal that would be present in the case of heterolytic cleavage, see outlook 6.

5.6.3 DPP-DMAP

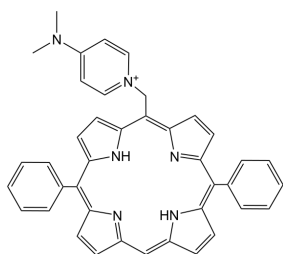


Figure 5.14: DPP-DMAP

The sample was again prepared in a mixture of $R \approx 1:100$ with CHCA as the matrix and DMSO as the solvent for MALDI. Samples were irradiated with 290 fs laser pulse duration and pulse energies of $E_{343} \approx 13 \mu\text{J}$ focused to a fluence of $F = 3.67 \text{ mJ}/\text{cm}^2$ to obtain mass spectra for neutrals and ions. Neutral particles were studied by deflecting all charged particles with electrode voltages up to 142 V and using the F₂ laser for postionization. The highest particle yield was achieved by selecting the velocity class to $v \approx 571 \text{ m/s}$. The intact particle could not be detected by postionization, the peak at $m = 476 \text{ Da}$ was identified as the DPP core with an added CH₂ ($462 + 14 = 476 \text{ Da}$, figure 5.15 (1)). This suggests that the particle already cleaves during the desorption process at the connection of the tag and the F₂ laser postionizes the neutral fragments. It is however surprising that neutral fragments can be encountered, since a positive fragment would be expected in the case of a heterolytic cleavage process, which would imply the absence of the DPP fragment in the spectrum. The other two signals at $m = 621 \text{ Da}$ and $m = 665 \text{ Da}$ could point to sodium impurities which are not uncommon. DPP-DMAP plus one Na $597 + 23 = 620 \text{ Da}$ or plus three Na₃ $597 + 69 = 666 \text{ Da}$ would be approximately in the correct range. All peaks are depleted when irradiated by the OPO at 411 nm, which points to cleavage of a phenyl rings.

We could observe the main particle at $m = 595 \text{ Da}$ in the case of ions. Irradiation with the OPO at 411 nm resulted in both an increase in the main signal at 595 Da and the DPP+CH₂ fragment at $m = 476 \text{ Da}$ when the OPO irradiates the particles with 411 nm, figure 5.15 (2). The interpretation of this remains elusive, but it seems to indicate that at this wavelength, both the

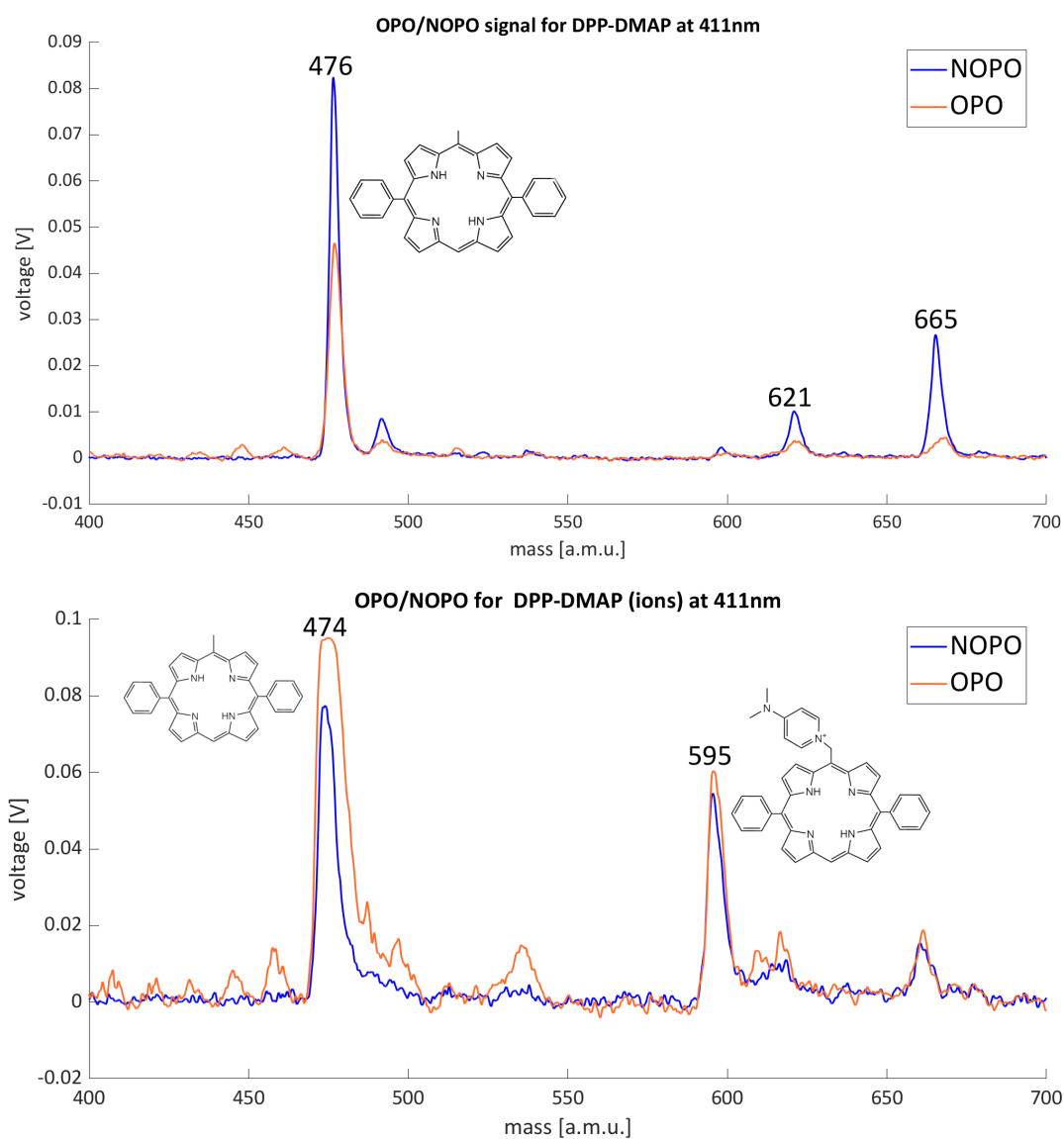


Figure 5.15: (1) Example of a mass spectrum for DPP-DMAP (neutrals) with and without OPO radiation (1.7 mJ). (2) Example of an ion signal with and without OPO radiation (1.7 mJ).

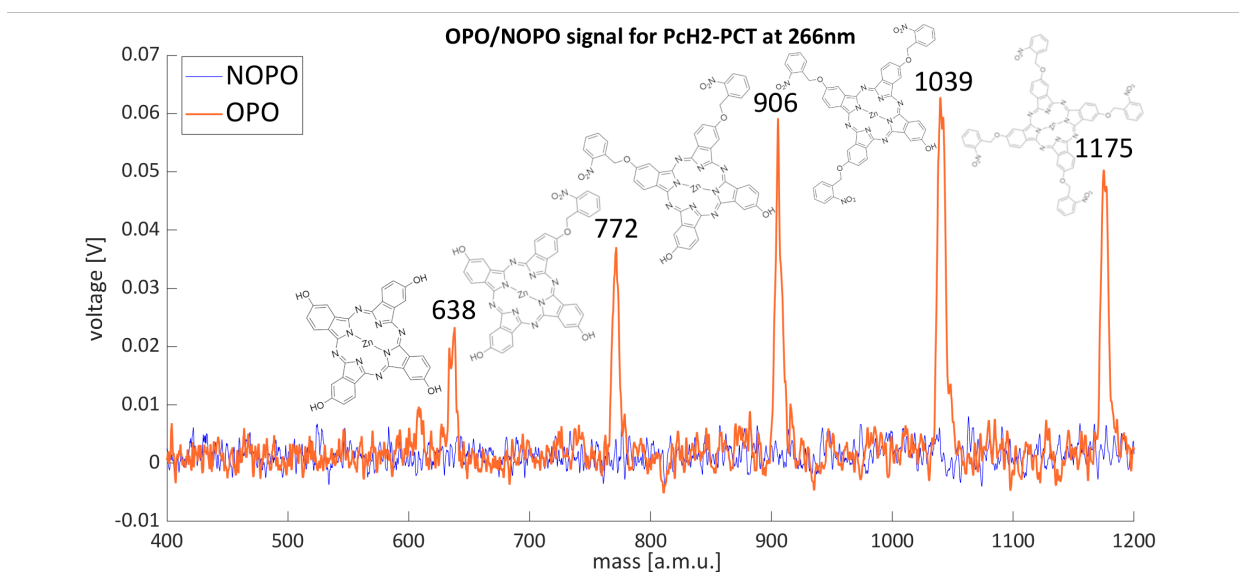


Figure 5.17: Mass spectrum for neutrals with and without 266 nm OPO irradiation. No signal is recorded in the absence of the OPO, since all ions are deflected. Irradiation of the OPO results in detection of the intact molecule and fragments that have cleaved off one up to four nitrobenzyl groups (here H-transfer).

main peak as well as the DPP fragment are postionized by 411 nm radiation. The photon energy of $E_\gamma = 3.02$ eV would imply a multi photon process. This is contradictory to the behaviour observed for neutrals.

5.6.4 Pch2-PCT

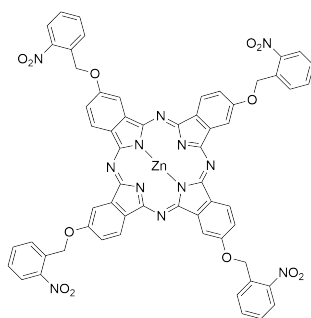


Figure 5.16: Pch2-PCT

The mixture prepared on the target wheel contained 4 μ l of the sample solution (66 mg/ml in DMSO) and 40 μ l of CHCA solution (120 mg/ml in DMSO) to obtain a sample/matrix ratio of $R \approx 1:100$ on the wheel. The sample was desorbed with 4 ns at 337 nm and focused to a fluence value of $F = 15$ mJ/cm². The velocity was selected to $v \approx 500$ m/s. Experiments at 676 nm were carried out, but neither cleavage or ionization of any kind could be detected at this wavelength. The photon energy of $E_\gamma = 1.83$ eV is probably too low to trigger any cleavage process, despite the high absorption for this wavelength.

Postionization and cleavage was detected for 266 nm. We studied neutral particles from the source and solely used the OPO at 266 nm to test for cleavage and postionization. The mass spectrum in figure 5.17 shows that in the absence of the OPO no signal could be detected since only neutrals pass through. With OPO radiation we clearly detect five peaks that can be assigned to the main molecule and fragments that cleaved off one, two, three or all four nitrobenzyl groups. However there is a small discrepancy between the expected mass spectrum and the observed one. If the nitrobenzyl group cleaves off without H-transfer (homolytically or heterolytically) at the location of the oxygen, it has an approximate mass of $(C_7H_6NO_2)$ $m = 136$ Da. If cleavage occurs under H-transfer (figure 4.2), the mass is $(C_7H_5NO_2)$ $m = 135$ Da.

We observe $m_0 = 1175$ Da, $m_{-1} = 1039$ Da, $m_{-2} = 906$ Da, $m_{-3} = 772$ Da and $m_{-4} = 638$ Da and corresponding discrepancies of $\Delta m_{0-1} = 136$ Da, $\Delta m_{1-2} = 133$ Da, $\Delta m_{2-3} = 134$ Da, $\Delta m_{3-4} = 134$ Da. Only the first mass difference matches a cleavage process without H-transfer, the other mass discrepancies differ by 1 or 2 mass units for cleavage under H-transfer and 2-3 mass units for cleavage without H-transfer. This discrepancy can be ascribed to two factors: First, the resolution of the TOF-MS (1/500) limits the determination of all mass peaks to an accuracy of about 2 to 1 Da. Second, the shift in the mass spectrum from the expected mass of around 1180 Da to 1175 Da for the main particle indicates a slight miss calibration of the TOF-MS. Despite these minor differences, the observed mass discrepancies still strongly suggest that we are detecting the intact molecule and the fragments that result from detachment of one up to four nitrobenzyl groups.

We observed in previously captured ion spectra that the molecule already fragments at the desorption stage to some degree. We therefore conclude that a large fraction of the signal after OPO irradiation is postionization of neutral fragments that come from the source. However, cleavage pathways from higher mass peaks into lower ones also need to be taken into account. A quantitative analysis of all processes is therefore a highly complicated problem and requires a system of coupled differential rate equations that takes both postionization and cleavage into account. The analysis is further complicated because occasional clustering of (PcH₂-PCT)₂ with a mass of $m = 2360$ Da that was also observed, and the resulting fragmentation from the clusters into the lower mass peaks must also be incorporated into a rate equation model. This makes a quantitative analysis even more difficult, which is the reason why we restrict ourselves to a qualitative discussion here. The observed response at 266 nm makes this particle a promising candidate for the grating mechanism. The exact process that depletes the molecules from the beam in a photodepletion grating is only of minor relevance, it can be ionization or fragmentation or also a combination of both. The observed results seem to indicate that indeed both play a role. The molecule is therefore an interesting candidate to combine a protein with and test again for ionization/fragmentation at 266 nm. Given that the particle can be desorbed at a moderate speed of 400 m/s, it would be beneficial to conduct interferometry solely with this particle before applying the method to proteins. This would allow to verify the functionality of the diffraction mechanism and calibrate the interferometer accordingly.

In summary, it can be said that of all particles studied, the PcH₂-PCT is the most promising candidate for interferometry, as it shows signs of cleavage and post-ionization at 266 nm, which, as we have discussed above, is the preferred wavelength for the grating mechanism. To determine the exact nature of the cleavage process, it is necessary to establish even softer desorption conditions to detect only the intact main particle. After irradiation, the mass spectrum should indicate whether it is a homolytic or heterolytic process, or cleavage under H-transfer. For these tests, a more precise TOF-MS would be advantageous, which can measure mass differences from one H atom up to 5000 Da and can change the polarity, see the outlook (chapter 6) below.

Despite their resonance in the visible range (422 nm for TPP and 411 nm for DPP-TRP and DPP-DMAP) that makes the porphyrins appear promising for neutralization and postionization, the tests conducted so far suggest that they are not suitable. Both TPP and DPP-TRP exhibited only homolytic behavior, which is not compatible with neutralization/postionization processes that require charge transfer. Moreover, these appear to be 2 photon processes, which lowers efficiency. The off resonant 2photon ionization for TPP at 266 nm could be used for diffraction, but a 1 photon process would be preferable. The cleavage behavior of DPP-DMAP remains undetermined so far. Further testing with a more precise TOF-MS and variations in the desorption conditions may provide additional insights. However, for neutralization and postionization at 532

nm, the already successfully tested bodipy 2-OH tag from the section [4.2.2](#) would be a promising alternative. Also, the PcH2-PCT particle at 266 nm could be used for postionization.

Chapter 6

Outlook

The measurements in the previous chapter revealed two main constraints for the interpretation of mass spectra and cleavage behaviour: the relatively low TOF resolution of 1/500 and the detection limited to positive ions. We are therefore adapting the setup to a different TOF-MS that is equipped with a reflectron and the ability to switch between positive and negative detection mode. The reflectron boosts the resolution 10 times to 1/5000, which allows unambiguous identification of masses that differ by only 1 mass unit and processes like H-transfer up to 5 kDa. This enables a more detailed investigation of cleavage processes in combination with the negative detection mode, as for example negative fragments that can result from a heterolytic cleavage process can be directly verified in contrast to the case above. In addition, higher resolution is necessary for experiments with high-mass proteins tagged with PCTs.

We will additionally exchange the wheel with a plate source to improve on sample efficiency and to allow simultaneous tests of different analyte/sample concentrations to determine the most suitable one. This was not possible with the wheel since no individual spots could be targeted with continuous rotation. Furthermore, the wheel method required coating of the total surface, which is not the most economical solution for PCTs with limited amount of sample to test. The plate source will be remotely controlled and mobile in the xy-plane, to enable the selection of different spots by the laser. Spots with different concentrations and crystallization on stainless steel plates are a standard method in MALDI based applications [99].

Finally, we want to briefly look at recent developments for superconducting detectors that could be applied to matter-wave interferometry. So far we saw that a frequently used detection scheme for matter-wave interferometers is the acceleration and separation of m/z -values of postionized molecules in a TOF-MS and subsequent detection with a secondary electron multiplication (SEM) unit like MCPs. Quadrupole mass spectrometers with SEMs are also frequently used in mass spectrometry [171]. SEMs rely on high kinetic energy of the molecules for efficient detection and quantum ion yields η are high for particles like electrons $\eta \approx 90\%$ [172], however proteins with masses beyond 20 kDa require acceleration energies of 40 keV [173]. Mass and structure also influence detection efficiency [174]. Higher m/z values that would increase the detection efficiency are disadvantageous because the PCTs considered so far only change charge values in terms of $1e$ and mass spectra are additionally harder to interpret with multiple charged species.

Superconducting nanowire detectors (SNWDs) or superconducting single particle detectors (SSPDs) are a promising alternative to conventional TOF-MS. SSPDs are well known in photonics and have recently been investigated as detectors for biomolecules that accept kinetic energies about a 100 times smaller than typical SEM detectors [175]. The superconducting 'wire' is typically etched on a silicon substrate in a meander form, see figure 6.1. The detection principle can be

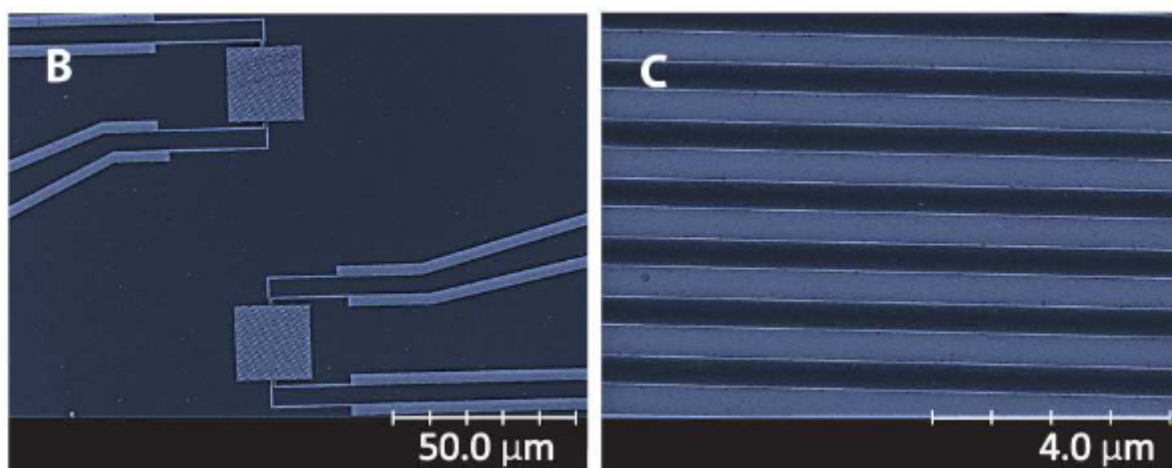


Figure 6.1: Electron micrograph snapshot of SSPD pixels. B: two pixels with a size of $20 \times 20 \mu\text{m}^2$. C: Close up image of one pixel. Figure adapted from [175].

illustrated as follows: the cooled superconducting nanowire ($\approx 3 \text{ K}$) is driven by a bias current I_b of 10-100 μA . An impinging particle can break up Cooper pairs and causes a transition from the superconducting state to normal conductivity. This is known as the local hotspot model. The induced resistance further extends over the whole width of the strip once a critical current density is overcome. Additional continuous supply of current into the resistive area creates fast voltage peaks along a shunt resistor that creates the detection signal [175]. Thermal relaxation resets the detector.

In the experiment, the detection efficiency of the SNWDs was compared to conventional TOF-MCP detection and detection by a phosphor screen coupled to a photon multiplier (PhS). Different types of proteins were launched in an ESI source, with the possibility for charge reduction in a Corona discharge chamber and subsequent mass filtering in a QMS, before being guided to either the TOF-MS plus MCP, the PhS or the SSPD. The SSPDs detected identical mass spectra at significantly lower kinetic energies. Mass spectra of the protein concanavalin A were recorded with impact energies 50 to 200 lower than for SEM devices, the acceleration voltage was $U \leq 190 \text{ V}$ for the SSPDs, whereas $U = 10 \text{ kV}$ were needed for PhS and $U = 13 \text{ kV}$ for the MCP. Additional measurements were conducted with a protein mix of singly and doubly charged insulin, cytochrome C and myoglobin that further demonstrated the high sensitivity of the detectors. Singly charged myoglobin was detected with 200 times lower impact energy compared to the other detectors. The low energies are intriguing since they allow detection of particles with velocities of 200-500 m/s and below [175], which would make postionization obsolete since no additional acceleration in a TOF-MS is required. This would simplify the interferometer design tremendously. The detector chips built in the experiment used 32 pixels of size $A_1 = 20 \times 20 \mu\text{m}^2$ (figure 6.1), or ten pixels of $A_2 = 200 \times 200 \mu\text{m}^2$. The simultaneous use of hundreds or thousands of such chips would be an exciting new detection method for low kinetic energy molecular beams and high mass molecules with low charge states, which could prove advantageous for future matter wave experiments.

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Appendix A

Higher frequency generation and non-linear optics

The laser systems used in the experiments utilize non-linear optical elements to generate laser light with double, triple and higher frequency values of the fundamental frequency ω_0 , being referred to as second harmonic generation (SHG) for $2\omega_0$, third harmonic generation for $3\omega_0$ (THG) and higher harmonic generation (HHG), respectively. Proper mixing of different frequencies in non-linear crystals can additionally be used to smoothly change the output frequency or wavelength of the laser system through a wide range of values. This section will briefly describe the physical models behind these techniques, necessary to understand the laser systems used in our experiments.

Non-linear optics

The polarization density $\mathbf{P}$ of some dielectric medium is a vector valued quantity that describes the dipole moment $\mathbf{p}$ per volume $\mathbf{V}$ [176]:

$$\mathbf{P} = \frac{d\mathbf{p}}{dV} \quad (\text{A.1})$$

Polarization of a medium can be created by an external electric field $\mathbf{E}$ that induces dipole moments in the atoms of the dielectric. For time varying electromagnetic fields, these induced dipole moments and hence the polarization also vary in time, which in return leads to the emission of electromagnetic radiation by the oscillating atoms. For small intensities of the incident field, the relationship between the electric field and the induced polarization density is linear, however for higher values, the relationship can be expanded into a Taylor series [167]:

$$\mathbf{P} = \epsilon_0(\chi_1 \mathbf{E} + \chi_2 \mathbf{E}^2 + \dots) \quad (\text{A.2})$$

where $\epsilon_0 \approx 8.854 \cdot 10^{12} \text{ F/m}$ is the vacuum permittivity. In general, second order effects only arises in non-isotropic media like crystals, implying that the polarization density is no longer directly proportional to the electric field and the i^{th} order susceptibilities are tensors [177]:

$$\frac{P_i}{\epsilon_0} = \sum_j \chi_{ij}^{(1)} E_j + \sum_{jk} \chi_{ijk}^{(2)} E_j E_k + \dots \quad (\text{A.3})$$

This equation describes the basic condition for creating higher order harmonics. Consider an incident plane wave with two different components ($i=1,2$) [167]:

$$E_i = \frac{A_i}{2} \exp[i(\mathbf{k}_i \mathbf{r} - \omega_i t)] + c.c \quad (\text{A.4})$$

with the wave vector $\mathbf{k}_i = 2\pi n_i/\lambda$, where n_i is the refractive index in direction of the electrical field component. For the second order susceptibility χ_2 in the case of direct proportionality, inserting Eq. A.4 into Eq. A.2 leads to a polarization density $\mathbf{P}(\omega, \mathbf{k})$, where ω and $\mathbf{k}$ can be of the form [167]:

$$\omega = \omega_1 \pm \omega_2; 2\omega_1; 2\omega_2 \quad (\text{A.5})$$

$$\mathbf{k} = \mathbf{k}_1 \pm \mathbf{k}_2; 2\mathbf{k}_1; 2\mathbf{k}_2 \quad (\text{A.6})$$

So under proper conditions, one can for example achieve frequency doubling via $\omega = 2\omega_0$, or generating the third harmonic by mixing of the fundamental with the second harmonic $\omega = \omega_0 + 2\omega_0 = 3\omega_0$. In order to achieve a high amplification of the generated signal, it is important to ensure that the waves travel through the crystal with equal phase velocity, a condition referred to as phase matching:

$$|\mathbf{k}| = \frac{2\pi n}{\lambda} = |2\mathbf{k}_i| = \frac{4\pi n_i}{\lambda} \quad (\text{A.7})$$

Therefore, to fulfill the phase matching condition, the two waves need to propagate along directions with equal indices of refraction $n = n_i$.

Parametric amplification and oscillation

The conservation of energy and momentum described by equations A.5-A.6 is the basic physical principle for various applications using non-linear optics. Equation A.5 implies a process where a high-intensity 'pump' beam ω_p interacts with a lower-intensity 'signal' wave ω_s within a non-linear crystal. This interaction generates a new wave, referred to as the 'idler' wave ω_i , whose frequency is the difference between the pump and signal frequencies $\omega_i = \omega_p - \omega_s$. Subsequently, this new formed idler wave interacts again with the pump wave within the crystal, producing additional light at the frequency of the original signal wave: $\omega_s = \omega_p - \omega_i$. If the phase matching condition $|\vec{k}_s| = |\vec{k}_p| - |\vec{k}_i|$ is full filled, the result is an overall amplification of the signal wave at expense of the pump intensity. The phase matching condition depends on the polarization of the waves and the orientation of the crystal. If the signal and idler wave have the same polarization, the process is called type I phase matching. If they are perpendicular, it is called type II phase matching (for a precise mathematical treatment of this problem see for example: [177]). Additionally, if the crystal is put into an optical cavity that resonates the signal and/or idler wave, they can perform oscillations, where each round trip results in further amplification of the resonant waves, compensating for possible losses by mirror imperfections, etc. Such a device is called an optical parametric oscillator (OPO). Since the parametric amplification described above depends on the incident pump power, a certain threshold can be reached where the amplification in the OPO system exceeds the losses and, using a partly transmitting mirror, a strong signal of the resonant wave in the OPO can be created. Generation of different frequencies is achieved by varying the phase matching Eq. A.7 with tilted, rotated or induce stress in birefringent crystals [167]. This technique enables OPO systems to cover a large bandwidth of electromagnetic radiation. In combination with higher harmonic generation, complex laser systems can be built.

A subtle detail in the OPO process is the generation of the initial photons for the signal and idler waves via spontaneous photon down conversion, which is a non classical process induced by the quantized electromagnetic field. In the crystal, a photon of the pump beam can be converted into two photons with different frequencies when energy and momentum conservation

(Eq. A.5-A.6) are respected, by population of the respective vacuum modes:

$$|1\rangle_p |0\rangle_s |0\rangle_i \rightarrow a_p a_s^\dagger a_i^\dagger |1\rangle_p |0\rangle_s |0\rangle_i = |0\rangle_p |1\rangle_s |1\rangle_i \quad (\text{A.8})$$

where a_p , $a_s^\dagger$, $a_i^\dagger$ are the annihilation and creation operators for the respective modes. This process can be thought of as stimulated emission by the vacuum and happens with very low efficiency, but it acts as a triggering event for the optical parametric oscillation.

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